

Award Number:

W81XWH-10-1-0444

TITLE:

**Assessment of the Prognostic and Treatment-Predictive Performance of the Combined HOXB13:IL17BR-MGI Gene Expression Signature in the Trans-ATAC Cohort**

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REPORT DATE: December 2013

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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| REPORT DOCUMENTATION PAGE   |             |                         |                            | Form Approved<br>OMB No. 0704-0188                    |   |
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| 1. REPORT DATE<br>December 2013   |             | 2. REPORT TYPE<br>Final |                            | 3. DATES COVERED<br>1 September 2010-30 November 2013 |   |
| 4. TITLE AND SUBTITLE<br>Assessment of the Prognostic and Treatment-Predictive Performance of the Combined HOXB13:IL17BR-MGI Gene Expression Signature in the Trans-ATAC Cohort   |             |                         |                            | 5a. CONTRACT NUMBER                                   |   |
|   |             |                         |                            | 5b. GRANT NUMBER<br>W81XWH-10-1-0444                  |   |
|   |             |                         |                            | 5c. PROGRAM ELEMENT NUMBER                            |   |
| 6. AUTHOR(S)<br>Dennis Sgroi M.D.<br><br>email: dsgroi@partners.org   |             |                         |                            | 5d. PROJECT NUMBER                                    |   |
|   |             |                         |                            | 5e. TASK NUMBER                                       |   |
|   |             |                         |                            | 5f. WORK UNIT NUMBER                                  |   |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br>Massachusetts General Hospital<br>Boston, MA 02114  |             |                         |                            | 8. PERFORMING ORGANIZATION REPORT NUMBER              |   |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012   |             |                         |                            | 10. SPONSOR/MONITOR'S ACRONYM(S)                      |   |
|   |             |                         |                            | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)                |   |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT<br>Approved for Public Release; Distribution Unlimited  |             |                         |                            |   |   |
| 13. SUPPLEMENTARY NOTES   |             |                         |                            |   |   |
| 14. ABSTRACT<br>We compared the prognostic ability of the Breast Cancer Index (BCI), Oncotype DX Recurrence Score (RS) and IHC\$ for both early and late recurrence among patients with ER+, node negative (N0) disease with the ATAC clinical trial. BCI was performed from 1102 primary tumor samples from ER+ patients were evaluated. BCI-L, IHC4 and RS had significant prognostic performance for early DR (BCO-L, p<0-0002), while only BCI-L was significant for late DR (LR-ΔX2: 7-97, p=0-0048). For risk of early DR at 5 years, BCI-L classified 59%, 25%, and 16% of patients with 1.3%, 5.6%, and 18.1% for low, intermediate and high risk, respectively. For risk of late DR at 10 years, BCI-L classified 61%, 25%, and 14% of patients.   |             |                         |                            |   |   |
| 15. SUBJECT TERMS- HOCB13?IL17BR(H/I), Molecular Grade Index (MGI), Breast Cancer Index (BCI), estrogen receptor-positive breast cancer, TransATAC  |             |                         |                            |   |   |
| 16. SECURITY CLASSIFICATION OF:   |             |                         | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES                                   | 19a. NAME OF RESPONSIBLE PERSON           |
| a. REPORT   | b. ABSTRACT | c. THIS PAGE            |                            |   | USAMRMC                                   |
| U   | U           | U                       | UU                         | 28  | 19b. TELEPHONE NUMBER (include area code) |

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## **Final Report : BC097711 - “Assessment of the Prognostic and Treatment-Predictive Performance of the Combined HOXB13:IL17BR-MGI Gene Expression Signature in the Trans-ATAC Cohort”**

**PI: Dennis Sgroi M.D.**

### **BACKGROUND**

Greater than 50% of recurrences in estrogen receptor-positive (ER+) breast cancer occur after 5 years of adjuvant endocrine therapy. Biomarkers capable of improving the risk-benefit of extended adjuvant endocrine therapy for these late recurrences would be clinically valuable. We compared the prognostic ability of the Breast Cancer Index (BCI), Oncotype DX Recurrence Score (RS) and IHC4 for both early and late recurrence among patients with ER+, node negative (N0) disease within the ATAC clinical trial.

### **METHODS**

BCI was performed from 1102 primary tumor samples from ER+ patients and two versions (BCI-C (primary) and BCI-L (secondary), based on cubic and linear combinations of the variables) were evaluated. RS and IHC4 values were previously derived. Prognostic discrimination for early (<5y) and late recurrence (5-10y) was assessed. To evaluate the ability of the biomarkers to predict recurrence beyond standard clinicopathological parameters, the likelihood-ratio chi-square ( $LR-\Delta\chi^2$ ) was calculated from Cox proportional hazards models. The primary endpoint was distant recurrence (DR).

### **FINDINGS**

In the primary analysis of 665 ER+ N0 patients, categorical BCI-C demonstrated significant differences in risk of DR over 10 years ( $P<0.0001$ ). In the secondary analysis, BCI-L proved to be a much stronger predictor, and BCI-L, IHC4 and RS had significant prognostic performance for early DR (BCI-L,  $p<0.0002$ ), while only BCI-L was significant for late DR ( $LR-\Delta\chi^2: 7.97, p=0.0048$ ). For risk of early DR at 5 years, BCI-L classified 59% (390/665), 25% (166/665) and 16% (109/665) of patients with 1.3% (0.5% - 3.1%), 5.6% (2.9% - 10.5%) and 18.1% (12.0% - 27.0%) for low, intermediate and high risk, respectively. For risk of late DR at 10 years, BCI-L classified 61% (366/596), 25% (146/596) and 14% (84/596) of patients with 3.5% (2.0% - 6.1%), 13.4% (8.5% - 20.8%) and 13.3% (7.4% - 23.4%) for low, intermediate and high, respectively.

### **INTERPRETATION**

While all three biomarkers predicted for early DR, BCI-L was the only significant prognostic for risk of late DR. The three BCI-L groups identified two risk populations for both early and late DR with 84% (556/665) of patients having low risk for early DR, and a smaller population (39%, 230/596) having high risk for late DR who may benefit from extended endocrine or other therapy.

*Task 1.* To convert RNA from 1231 TransATAC tumor samples into cDNA (Months 1-9 months):

- a. DNase treatment of 1231 tumor RNA samples (Months 1-5).

- b. Conversion of 1231 tumor DNase-treated RNA samples into cDNA (Months 6-10).
- c. cDNA purification (Months 11-12).

**Progress report for Task 1:** First have completed task 1 in which RNA from 1072 TransATAC tumor samples has been successfully converted in cDNA and purified.

*Task 2.* To perform the HOXB13:IL17BR+MGI real-time quantitative PCR analysis (Months 13-19):

- a. Real-time PCR analysis of the four normalizing genes (Months 13-15). Triplicate reactions for each gene for 1231 samples.
- b. Real-time PCR analysis of HOXB13:IL17BR and MGI (Months 16-19). Triplicate reactions for all 7 genes for 1231 samples.

**Progress for Task #2:** We have now completed Task #2 in which we have performed our real-time PCR analysis for four normalizing genes, for HOXB13:IL17 (H/I) and for MGI on all 1072 TransATAC samples. For our analysis, which includes a comparison to Oncotype Dx and IHC4 (ER, PR< HER2 and Ki-67), we excluded 157 samples as IHC4 was not available for these samples. Thus, we have a final cohort size of 915 patients (665 patients are LN- and 250 are LN+) for which we have cDNA that passed quality control analysis and for which we have OncotypeDx and IHC4 data for comparison.

*Task 3.* Assessment of the prognostic and predictive performance of HOXB13:IL17BR-MGI, comparative analysis with Oncotype Dx, and manuscript preparation.

- a. Perform statistical analysis for specific aims 1 through 4 (Months 20-24).
- c. Manuscript preparation (Months 22-24).

**Progress for Task #3:** The primary objective of the study was to first determine the prognostic power of the HOXB13:IL17BR (H/I), MGI and the combined biomarker (Breast Cancer Index , BCI) in LN- TransATAC patients. As can be seen in Table 1, MGI and H/I alone are prognostic in the univariate model with HR values of 2.26 and 2.33 ( $p < 0.0001$ ), respectively. Furthermore, they remained prognostic in the multivariate model (+CTS, clinical treatment score which consists of tumor size and tumor grade). As expected, the combination of the two biomarkers as the breast cancer index (BCI), provided additional robust prognostic power with an HR value of 3.12 and 2.30 in the univariate and multivariate models, respectively.

## BCI (linear), MGI, H/I

|              | HR (95% CI)      | LR- $\chi^2$ (P-value) |
|--------------|------------------|------------------------|
| BCI (linear) | 3.12 (2.25-4.32) | 48.96 (<0.0001)        |
| +CTS         | 2.30 (1.62-3.27) | 22.69 (<0.0001)        |
| MGI          | 2.26 (1.66-3.08) | 28.04 (<0.0001)        |
| +CTS         | 1.62 (1.16-2.26) | 8.41 (0.004)           |
| H/I          | 2.33 (1.63-3.32) | 21.18 (<0.0001)        |
| +CTS         | 2.02 (1.43-2.88) | 15.34 (0.0001)         |

Table 1. Hazard ratio and LR- $\chi^2$  values for BCI, MGI and H/I in the 665 LN- breast cancer patients in the TransATAC cohort in a univariate and multivariate (+clinical treatment score, CTS) models.

A Kaplan-Meier analysis of distant recurrence as defined by BCI groups (low, intermediate and high risk) demonstrates a robust and statistically significant stratification of the LN- TransATAC patients (Fig. 1).

### Kaplan-Meier distant recurrence according to BCI (linear) group

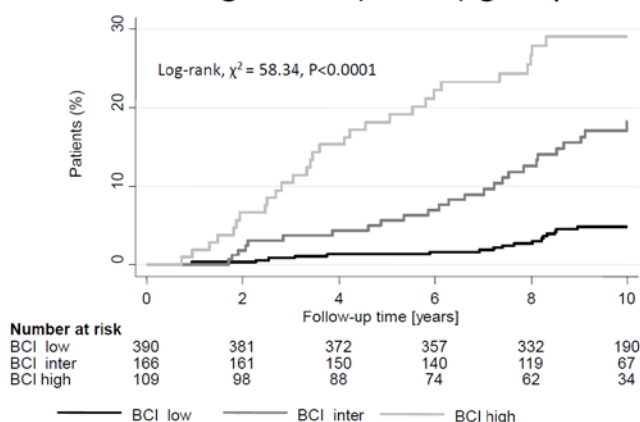


Figure 1. Kaplan-Meier plots of BCI as three predefined risk groups (BCI low, intermediate and high) in the 665 LN- breast cancer patients in the TransATAC cohort.

A direct head-to-head comparison of BCI, MGI and H/I with IHC and the OncotypeDx recurrence score (RS) is seen in Figure 2. In both the univariate and multivariate analyses, BCI and IHC have comparable prognostic power. Notably, BCI have nearly twice the prognostic power the Oncotype Dx RS in both the univariate and multivariate analyses (compare the LR- $\chi^2$  values which provide for a direct comparison).

### Prognostic information for distant recurrence in node-negative patients (N=665)

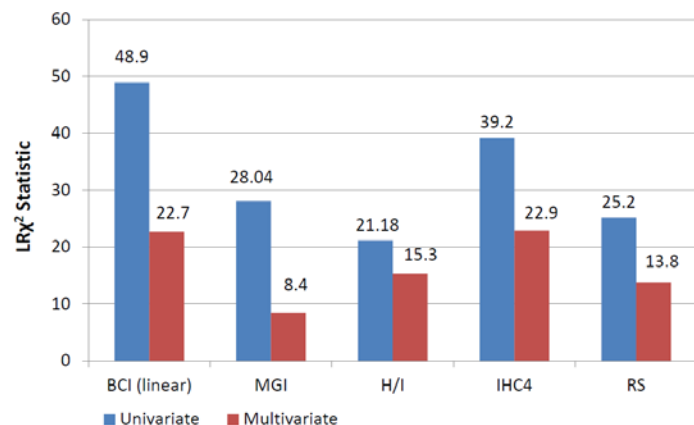


Figure 2. Comparison of BCI, MGI, H/I with IHC4 and the Oncotype Dx recurrence score (RS) in the 665 LN- TransATAC breast cancer patients

We next assessed the treatment-predictive performance of BCI, H/I and MGI. As demonstrated in Table 2, neither BCI nor MGI demonstrate a significant interaction with treatment. However, H/I demonstrates a significant difference in HR values for anastrozole and tamoxifen with a significant interaction term. This indicates that the H/I predicts for anastrozole benefit as compared with tamoxifen. Most, notably analysis of the OncotypeDx RS did not predict for treatment benefit (data not shown).

### BCI, MGI, H/I according to treatment arm (adjusted for CTS)

|               | HR (95% CI)      | LR- $\chi^2$ (P-value) |
|---------------|------------------|------------------------|
| <b>BCI</b>    |                  |                        |
| Anastrozole   | 1.88 (1.15-3.10) | 6.63 (0.01)            |
| Tamoxifen     | 2.82 (1.73-4.61) | 17.65 (<0.0001)        |
| P-interaction | 0.23             |                        |
| <b>MGI</b>    |                  |                        |
| Anastrozole   | 1.87 (1.17-3.01) | 7.06 (0.008)           |
| Tamoxifen     | 1.42 (0.89-2.27) | 2.24 (0.1)             |
| P-interaction | 0.44             |                        |
| <b>H/I</b>    |                  |                        |
| Anastrozole   | 1.19 (0.71-1.98) | 0.43 (0.5)             |
| Tamoxifen     | 3.28 (2.02-5.34) | 22.88 (<0.0001)        |
| P-interaction | 0.004            |                        |

Table 2. Hazard ratios for BCI, MGI and H/I per treatment arm in the TransATAC LN- breast cancer patients

At the time of this report, we have initiated several additional analyses of our biomarker performance in the LN+ breast cancer patients in the TransATAC cohort. Recently, we demonstrated in the MA.17 clinical trial of extended adjuvant hormonal that the H/I biomarker component of BCI predicts for late recurrence (i.e. recurrence that occur after 5 years from the time of diagnosis). Thus, we will assess if our biomarker performance is time-dependent by comparing the prognostic performance between 0 and 5 years, and

between 5 and 10 years post diagnosis. We anticipate that the H/I biomarker will predict for late recurrence in the TransATAC cohort.

In addition, we have initiated the analysis of the prognostic and treatment predictive performance of BCI, H/I and MGI in the 250 lymph node positive (LN+) TransATAC patients. We anticipate finishing these analyses in the next 3 month and completing manuscript preparation in the next 9-12 months.

Since the submission of our progress report in October of 2013, we have performed a more complete analysis of our findings as it relates to both the LN- and LN+ and have performed a comparative analysis to Oncotype Dx and IHC4. We are currently in the process of completing all of our analyses including a comparison to PAM50. Our findings to date are as follows:

#### Characteristics of the Study Population

Values for the RS, IHC4 and BCI were available for 915 women of whom 665 had ER-positive node-negative (N0) disease (Supplemental Figure 1). The clinical characteristics of these 665 patients are listed in Table 1, along with the characteristics of the 561 randomly assigned, ER-positive, N0 patients in the ATAC trial who were not included in this study. No statistically significant difference was observed except that the non-TransATAC cohort had more well differentiated tumors and fewer late distant recurrences than the TransATAC patients. In N0 women, there were 106 recurrences of which 72 were distant recurrences.

**Table 1.** Patient demographics and clinical characteristics in the present study and the broader population of node-negative patients in single-agent arms of ATAC trial.

|                    | N0 BCI cohort<br>TransATAC<br>(n=665) | N0 HER2- BCI<br>cohort<br>TransATAC<br>(n=597) | Non-TransATAC<br>UK N0 patients<br>(n=561) | P value* |
|--------------------|---------------------------------------|--|--|----------|
| Age, mean (SD)     | 63.3 (8.1)                            | 63.4 (8.0)                                     | 62.6 (7.8)                                 | 0.1      |
| BMI, mean (SD)     | 27.1 (4.8)                            | 27.2 (4.8)                                     | 26.8 (5.1)                                 | 0.3      |
| Tumor size         |                                       |  |  | 0.13     |
| <2 cm              | 486 (73.1%)                           | 442 (74.1%)                                    | 432 (77.0%)                                |          |
| 2-3 cm             | 144 (21.7%)                           | 125 (20.9%)                                    | 95 (16.9%)                                 |          |
| >3 cm              | 35 (5.2%)                             | 30 (5%)  | 29 (5.2%)                                  |          |
| Unknown            | 0                                     | 0  | 5 (0.9%)                                   |          |
| Tumor grade        |                                       |  |  | 0.005    |
| Well               | 143 (21.5%)                           | 138 (23.1%)                                    | 155 (27.6%)                                |          |
| Moderate           | 395 (59.4%)                           | 357 (59.8%)                                    | 300 (53.5%)                                |          |
| Poor               | 127 (19.1%)                           | 102 (17.1%)                                    | 78 (13.9%)                                 |          |
| Unknown            | 0                                     | 0  | 28 (5.0%)                                  |          |
| Radiotherapy       |                                       |  |  | 0.95     |
| No                 | 220 (33.1%)                           | 189 (31.7%)                                    | 187 (33.3%)                                |          |
| Yes                | 445 (66.9%)                           | 408 (68.3%)                                    | 374 (66.7%)                                |          |
| Mastectomy         |                                       |  |  | 0.86     |
| No                 | 439 (66.0%)                           | 404 (67.7%)                                    | 374 (66.7%)                                |          |
| Yes                | 226 (34.0%)                           | 193 (32.3%)                                    | 187 (33.3%)                                |          |
| Distant Recurrence |                                       |  |  |          |
| Early (0-5 years)  | 33 (5.0%)                             | 21 (3.5%)                                      | 23 (4.1%)                                  | 0.56     |
| Late (5-10 years)  | 39 (5.9%)                             | 36 (6.6%)                                      | 12 (2.3%)                                  | 0.002    |

\*comparison is between N0 TransATAC versus N0 Non-TransATAC cohorts. t tests were used for age and BMI, proportional test based on normal approximation was used for distant recurrence, all others used Fisher's exact test. Abbreviations: ER, estrogen receptor; N0, node negative; BMI, body mass index; UK, United Kingdom

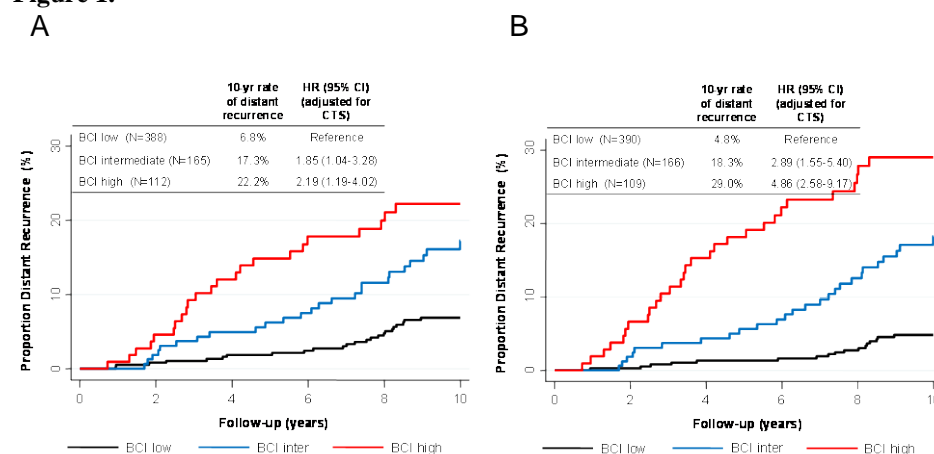


### BCI and Risk of Overall (0-10 years) DR

A cubic and a linear continuous model of BCI was previously developed using patients from the Stockholm clinical trial (). In the prespecified primary analysis using the BCI-cubic model (BCI-C) in all N0 patients, BCI-C was significantly associated with the overall (0-10 years) risk of DR (inter-quartile HR= 1.39; 95% CI, 0.99 to 3.70; LR- $\chi^2$  = 3.70; P =0.05) when adjusted for the effects of tumor size and grade, lymph node status, age and treatment as determined by the Clinical Treatment Score (CTS). Kaplan-Meier curves (Figure 1A) show clear differences in absolute DR rates according to prespecified BCI-C risk groups. The rates of DR at 10 years in the low, intermediate and high risk BCI-C groups were 6.8% (95% CI, 4.4% to 10.0%), 17.3% (95% CI, 12.0% to 24.7%), and 22.2% (95% CI, 15.3% to 31.5%), respectively. When adjusted for CTS, the HR between high and low BCI-C risk groups was 2.19 (95% CI, 1.19 to 4.02) and the HR between the intermediate and low risk groups was 1.85 (95% CI, 1.04 to 3.28) (Figure 1A).

Assessment of the pre-specified BCI-linear (BCI-L) model revealed that this model was also significantly associated with the overall risk of DR (inter-quartile HR= 2.30; 95% CI, 1.62 to 3.27; LR- $\chi^2$  = 22.69; P < 0.0001) when adjusted for CTS. Kaplan-Meier curves (Figure 1B) show clear differences in absolute DR rates according to prespecified BCI-L risk groups in which the low, intermediate and high risk BCI-L groups demonstrated rates of DR at 10 years of 4.8% (95% CI, 3.0% to 7.6%), 18.3% (95% CI, 12.7% to 25.8%), and 29.0% (95% CI, 21.1% to 39.1%), respectively. When adjusted for CTS, the HR between high and low BCI-C risk groups was 4.86 (95% CI, 2.58 to 9.17) and the HR between the intermediate and low risk groups was 2.89 (95% CI, 1.55 to 5.40) (Figure 1B). The overall 10 year risk of DR increased linearly with increasing BCI-L values in N0 patients (Supplemental Figure 2).

**Figure 1.**



**Figure 1.** Performance of pre-specified risk groups based on BCI-cubic and BCI-linear models for overall 10-year distant recurrences in all ER-positive N0 patients. A) BCI-cubic model; B) BCI-linear model.

In the HER-negative N0 subset, both BCI-C and BCI-L remained significantly associated with overall risk of DR with inter-quartile HR values of 1.65 (95% CI, 1.12 to 2.43; LR- $\chi^2$  = 6.61; P =0.01) and HR= 2.49 (95% CI, 1.68 to 3.68; LR- $\chi^2$  = 21.9; P <0.0001),

respectively. Kaplan-Meier curves of the prespecified risk groups for both models demonstrated distinct differences in absolute DR (Supplemental Figure 3).

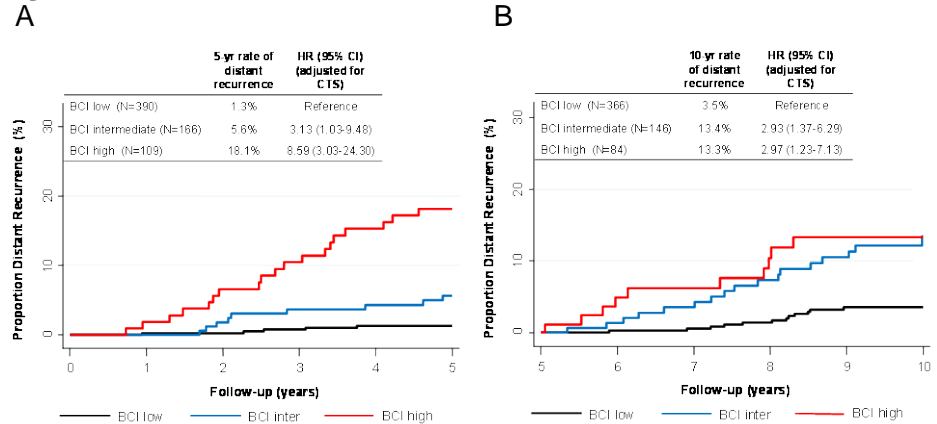
Comparison of the prognostic performance of BCI-L to BCI-C revealed that that BCI-L model was the strongest predictor of risk of recurrence. Thus, all subsequent analyses were performed utilizing BCI-L (henceforth referred to as BCI).

BCI and Risk of Early and Late DR

BCI was significantly associated with the risk of early (0-5 years) DR (inter-quartile HR= 2•77; 95% CI, 1•63 to 4•70; LR- $\chi^2$  = 15•42; P =0•0001, Table 2) when adjusted for CTS. Kaplan-Meier curves (Figure 2A) displayed significant differences in absolute DR rates at 5 years of 1•3% (95% CI 0•5% to 3•1%), 5•6% (95% CI 2•9% to 10•5%) and 18•1% (95% CI 12•0% to 27•0%) for the pre-specified low, intermediate and high BCI risk groups, respectively. Although three risk groups were pre-specified, the Kaplan-Meier analysis revealed that BCI identified two risk populations (Figure 2A). The first population (P1) consisted of BCI low and intermediate risk groups and constitutes 83% of all patients with a combined 5-year rate of DR of 2•6% (95%CI 1•5% to 4•3%). The second population (P2) consisted of the BCI high risk group and constitutes 17% of all patients with a 5-year rate of DR of 18•1%. When adjusted for CTS, the HR between the P1 and P2 was 4•61 (95% CI, 2•20 to 9•66).

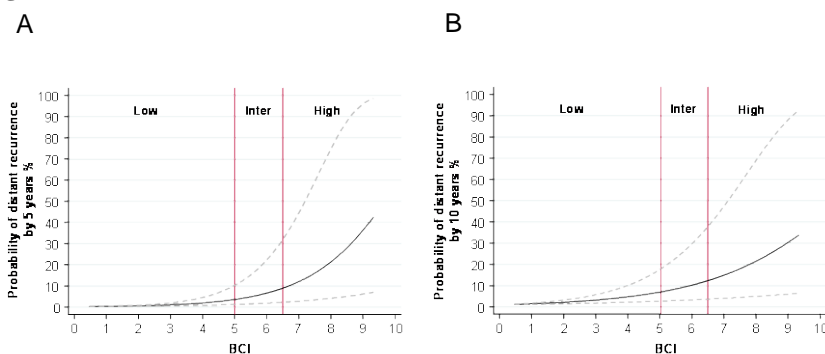
For late (5-10 years) recurrence, BCI was significantly associated with the risk of DR (HR= 1•95; 95% CI, 1•22 to 3•14; LR- $\chi^2$  = 7•97; P =0•005, Table 2) when adjusted for CTS. Kaplan-Meier curves (Figure 2B) displayed significant differences in absolute DR rates at 5 years of 3•5% (95% CI 2•0% to 6•1%), 13•4% (95% CI 8•5% to 20•5%) and 13•3% (95% CI 7•4% to 23•4%) for the low, intermediate and high BCI risk groups, respectively. The Kaplan-Meier analysis revealed two distinct risk populations in which the first population (P3) consists of the BCI low risk group and constitutes 61% of all patients with a 5-year rate of DR of 3•5% (Figure 2B). The second population (P4) consists of the BCI intermediate and high risk groups and constitutes 39% of all patients with a combined 5- year rate of DR of 13•4% (95%CI 9•3% to 19•0%). Adjusting for CTS, the HR between the low risk group (P3) and combined intermediate and high risk groups (P4) was 2•94 (95% CI, 1•44 to 6•01). The risk of DR increased linearly with increasing BCI values for both early and late recurrence (Figure 3).

Figure 2.



**Figure 2.** Performance of BCI pre-specified risk groups for early and late distant recurrences in ER-positive N0 patients. A) early 0-5 year distant recurrence; B) late 5-10 year distant recurrence. Population (P)1 refers to the pre-specified low and intermediate risk groups while P2, refers to the high risk group for early recurrence. P3 refers to the pre-specified low risk group, while P4 refers to the intermediate and high risk groups for late recurrence.

**Figure 3.**



**Figure 3.** Risk of early and late distant recurrence as a function of continuous BCI index in ER-positive N0 patients. A) risk of early 0-5 year distant recurrence; B) risk of late 5-10 year distant recurrence. Vertical lines delineate the borders between the low, intermediate (Inter) and high pre-specified BCI risk groups.

In the HER2-negative N0 subset, BCI was significantly associated with the risk of both early DR (inter-quartile HR = 3•26; 95% CI, 1•69 to 6•30; LR- $\chi^2$  = 13•53; P = 0•0002, Table 2) and late DR (inter-quartile HR = 2•12; 95% CI, 1•30 to 3•47; LR- $\chi^2$  = 9•45; P = 0•002, Table 2) and associated with distinct differences in absolute DR according to BCI risk groups (See Supplemental Figure 4). For both early and late recurrence the risk of DR increased linearly with increasing BCI values (Supplemental Figure 5).

**Table 2.** Comparative prognostic performance for early and late distant recurrence of BCI, RS and IHC4 in all node-negative patients and the node-negative HER2-subset.

|                                   |          | Early Recurrence( 0-5 Years) |                 | Late Recurrence (5-10 Years) |                 |
|-----------------------------------|----------|------------------------------|-----------------|------------------------------|-----------------|
|                                   |          | HR*                          | LR- $\chi^2$    | HR*                          | LR- $\chi^2$    |
|                                   |          | (95% CI)                     | (P-value)       | (95% CI)                     | (P-value)       |
| <b>UNIVARIATE</b>                 |          |                              |                 |                              |                 |
| BCI                               | N0       | 4.11 (2.52-6.70)             | 34.73 (<0.0001) | 2.47 (1.59-3.83)             | 16.55 (<0.0001) |
|                                   | N0 HER2- | 4.22 (2.32-7.64)             | 23.82 (<0.0001) | 2.84 (1.80-4.48)             | 20.61 (<0.0001) |
| RS                                | N0       | 1.96 (1.60-2.41)             | 28.89 (<0.0001) | 1.28 (0.95-1.72)             | 2.22 (0.1)      |
|                                   | N0 HER2- | 2.38 (1.61-3.53)             | 14.89 (<0.0001) | 1.59 (1.09-2.31)             | 5.02 (0.03)     |
| IHC4                              | N0       | 3.38 (2.39-4.78)             | 43.55 (<0.0001) | 1.55 (1.06-2.26)             | 4.79 (0.03)     |
|                                   | N0 HER2- | 4.08 (2.26-7.36)             | 20.74 (<0.0001) | 2.06 (1.29-3.28)             | 8.53 (0.004)    |
| <b>MULTIVARIATE INCLUDING CTS</b> |          |                              |                 |                              |                 |
| BCI                               | N0       | 2.77 (1.63-4.70)             | 15.42 (0.0001)  | 1.95 (1.22-3.14)             | 7.97 (0.005)    |
|                                   | N0 HER2- | 3.26 (1.69-6.30)             | 13.53 (0.0002)  | 2.12 (1.30-3.47)             | 9.45 (0.002)    |
| RS                                | N0       | 1.80 (1.42-2.29)             | 18.70 (<0.0001) | 1.13 (0.82-1.56)             | 0.51 (0.5)      |
|                                   | N0 HER2- | 1.93 (1.26-2.96)             | 8.23 (0.004)    | 1.28 (0.87-1.88)             | 1.45 (0.2)      |
| IHC4                              | N0       | 2.90 (2.01-4.18)             | 29.78 (<0.0001) | 1.30 (0.88-1.94)             | 1.63 (0.2)      |
|                                   | N0 HER2- | 3.41 (1.83-6.39)             | 13.89 (0.0002)  | 1.61 (0.98-2.66)             | 3.37 (0.07)     |

\*HR was calculated as between the inter-quartile range of the continuous scores of each biomarker.  
Abbreviations: BCI, Breast Cancer Index; RS, OncotypeDX recurrence score; IHC4, four immunohistochemical markers (estrogen receptor, progesterone receptor, human epidermal growth factor 2, and Ki-67; HR, hazard ratio; LR- $\chi^2$ ,  $\chi^2$  value based on the likelihood ratio statistic; CTS, clinical treatment score; N0, node negative; HER2-, epidermal growth factor receptor-negative

### H/I, MGI, and Risk of Early and Late DR

H/I has been demonstrated to predict for late DR in the MA.17 cohort.<sup>23</sup> Thus, we assessed H/I and MGI, the individual components of BCI, for their prognostic value for the risk of early and late DR. For early recurrence, MGI and H/I added significant prognostic information (Supplemental Table 3). However, for late recurrence only H/I provided additional information beyond standard clinicopathological factors (Table 3).

**Table 3.** Prognostic performance of H/I and MGI for early and late distant recurrence in hormone receptor-positive N0 patients.

| Early Recurrence (0-5 Years)      |                  |                        | Late Recurrence (5-10 Years) |                        |
|-----------------------------------|------------------|------------------------|------------------------------|------------------------|
|                                   | HR*(95% CI)      | LR- $\chi^2$ (P-value) | HR*(95% CI)                  | LR- $\chi^2$ (P-value) |
| <b>UNIVARIATE</b>                 |                  |                        |                              |                        |
| MGI                               | 3.15 (1.96-5.06) | 24.27(<0.0001)         | 1.74 (1.16-2.62)             | 7.26 (0.007)           |
| H/I                               | 2.42 (1.44-4.07) | 10.90 (0.001)          | 2.25 (1.38-3.66)             | 10.32 (0.001)          |
| <b>MULTIVARIATE INCLUDING CTS</b> |                  |                        |                              |                        |
| MGI                               | 2.10 (1.25-3.52) | 8.41 (0.004)           | 1.33 (0.86-2.06)             | 1.70 (0.2)             |
| H/I                               | 2.03 (1.20-3.41) | 7.05 (0.008)           | 2.02 (1.25-3.26)             | 8.17 (0.004)           |

\*HR was calculated as between the inter-quartile range of MGI and H/I.

Abbreviations: MGI, Molecular Grade Index; H/I, HOXB13/IL17BR gene expression ratio; HR, hazard ratio; LR- $\chi^2$ ,  $\chi^2$  value based on the likelihood ratio statistic; CTS, clinical treatment score; N0, node negative.

### Comparison of BCI with IHC4 and RS

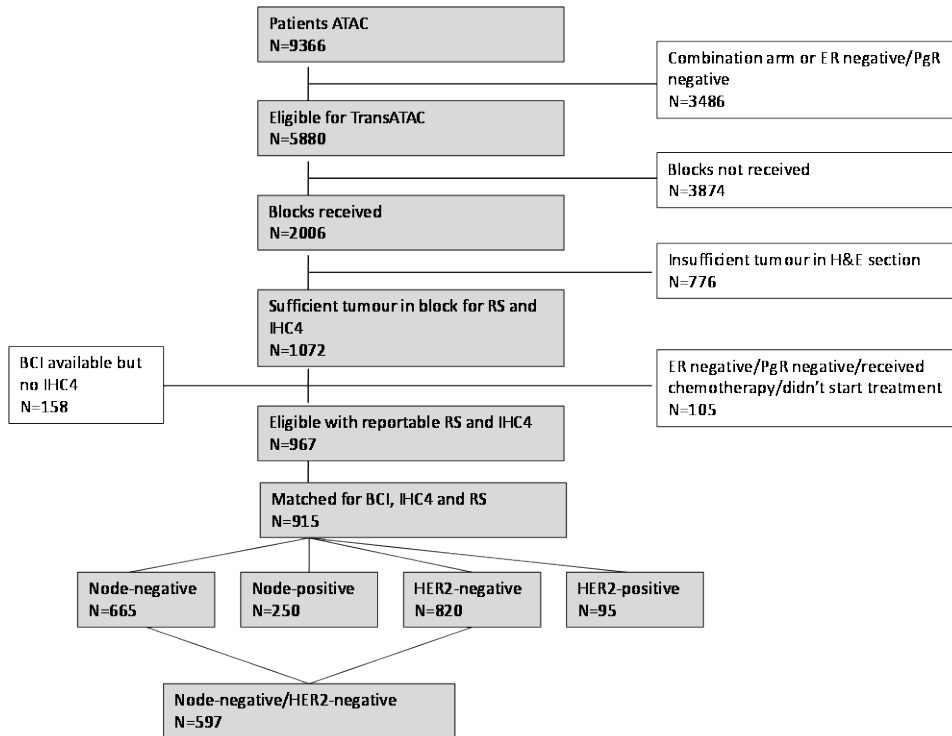
The likelihood ratio (LR- $\chi^2$ ) values were used to provide for a direct head-to-head comparison of BCI with IHC4 and RS. The relative prognostic performance of each biomarker varied depending upon the DR time frame (Table 2). For the early recurrence time frame, BCI, IHC4 and RS are all prognostic for DR in both univariate and multivariate analyses (Table 2). In all N0 patients, IHC4 was more prognostic than RS and BCI after adjusting for CTS. However, in the N0 HER2-negative patients, BCI and IHC4 demonstrated highly comparable prognostic performance that was superior to RS after adjusting for CTS. In the multivariate analysis of the late recurrence time frame, only BCI remained strongly prognostic in all N0 and N0 HER2-negative patients, while both IHC and RS were not prognostic in either population (Table 2). Similar results were observed considering all recurrences, breast cancer death and overall survival as end points (Supplemental Table 1).

Although the focus of this study centered on the N0 patients as the primary analysis population, an analysis of the N+ patients revealed that BCI was prognostic for the risk of

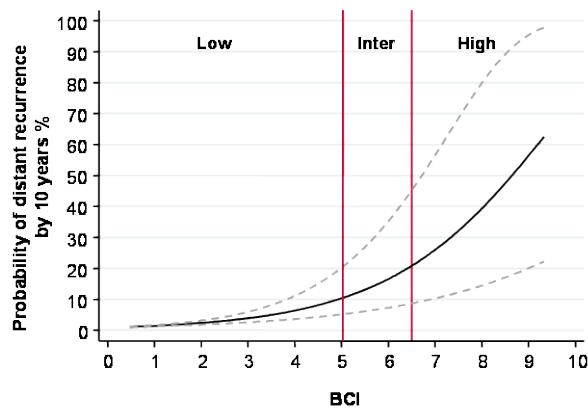
overall DR (Supplemental Figure 6, log rank  $p=0.005$ ). Furthermore, a comparative analysis revealed that BCI, IHC4 and RS had highly similar prognostic performance, albeit less robust than that observed in the N0 TransATAC subset (Supplemental Table 2). It is worthy of note, that the N+ patients in this study, unlike in parental ATAC trial, did not show any preferential benefit of anastrozole over tamoxifen (Supplemental Figure 7).

We hope to complete our final analyses in the next 2-3 months, and we expect to complete manuscript preparation and submission within the next 4-6 months.

## Supplemental Figures and Tables

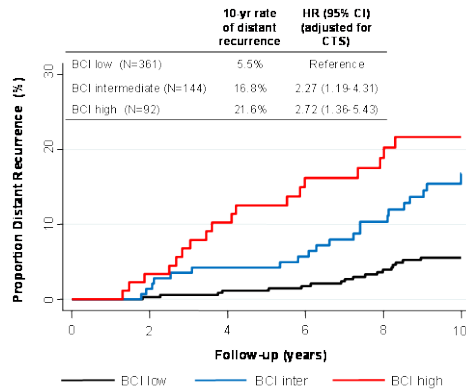


Supplemental Figure 1. ATAC CONSORT diagram.

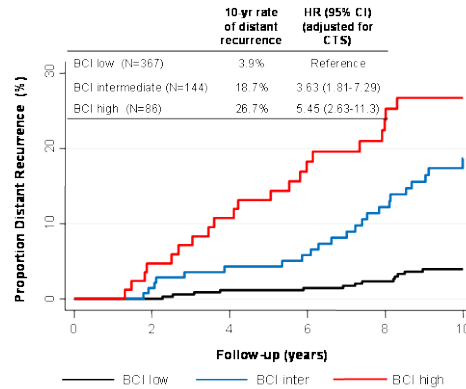


Supplemental Figure 2. Risk of overall 10-year distant recurrence as a function of continuous BCI-linear index in ER+ N0 patients.

A

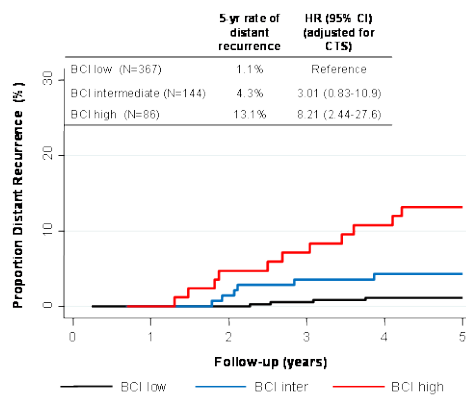


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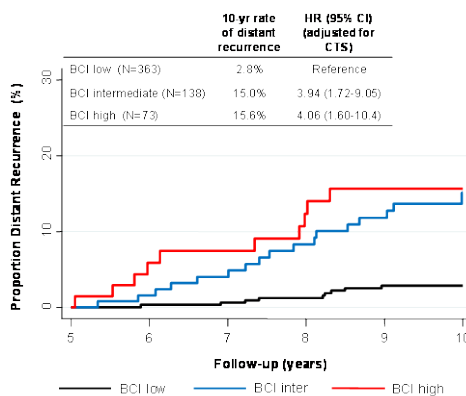


Supplemental Figure 3. Performance of pre-specified risk groups based on BCI-cubic and BCI-linear models for overall 10-year distant recurrences in ER-positive node-negative HER2- patients. A) BCI-cubic model; B) BCI-linear model.

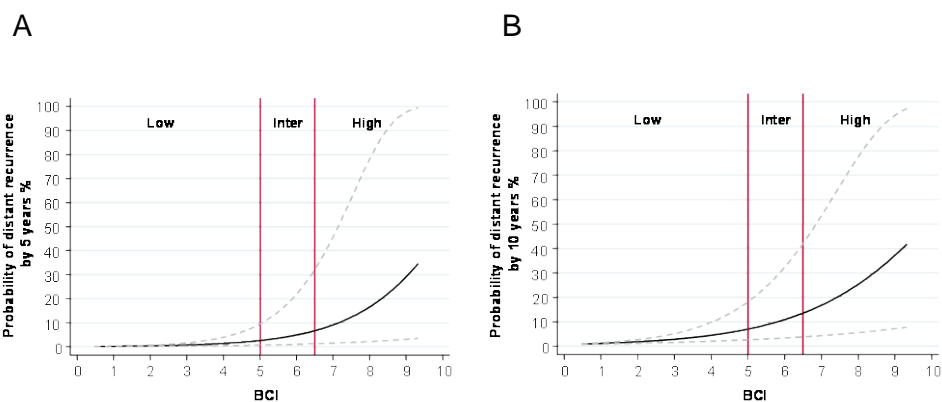
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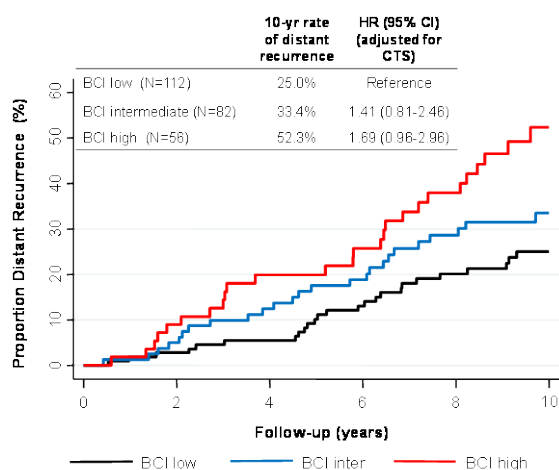
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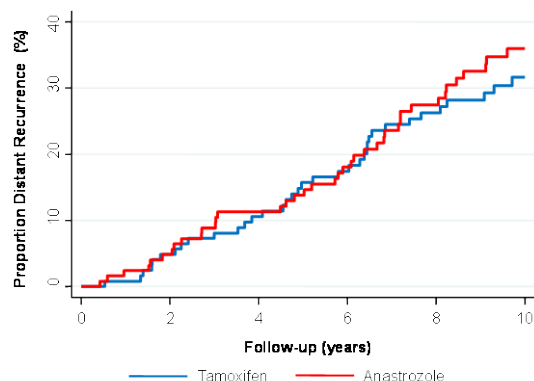
Supplemental Figure 4. Performance of BCI pre-specified risk groups for early and late distant recurrences in ER-positive node-negative HER2- negative patients. A) early (0-5 years) distant recurrence; B) late (5-10 years) distant recurrence.



Supplemental Figure 5. Risk of early (0-5 years) and late (5-10 years) distant recurrence as a function of continuous BCI index in ER-positive node-negative HER2- negative patients. A) risk of early distant recurrence; B) risk of late distant recurrence.



Supplemental Figure 6. Performance of pre-specified risk groups for overall 10-year distant recurrences in ER-positive node-positive patients.



Supplemental Figure 7. Distant recurrence free survival comparing anastrozole versus tamoxifen treated ER-positive node-positive patients.

Since our last interim progress report 5/12/13, we have completed all three proposed tasks, and our final analyses and data have been published in the Oct 14, 2013 issue of *The Lancet Oncology* (*Lancet Oncol.* 2013 Oct;14(11):1067-76.). The detailed descriptions that extend beyond what we reported in our previous interim report (5/12/13) can be reviewed in our *Lancet Oncology* article (see **Appendix**). In brief, our findings are as follows:

Our primary analysis showed significant differences in risk of distant recurrence over 10 years in the categorical BCI-C risk groups ( $p < 0.0001$ ) with 6.8% (95% CI 4.4–10.0) of patients in the low-risk group, 17.3% (12.0–24.7) in the intermediate group, and 22.2% (15.3–31.5) in the high-risk group having distant recurrence. Our secondary analyses showed that BCI-L was a much stronger predictor for overall (0–10 year) distant recurrence compared with BCI-C (interquartile HR 2.30 [95% CI 1.62–3.27]; LR- $\Delta\chi^2 = 22.69$ ;  $p < 0.0001$ ). When compared with BCI-L, the 21-gene recurrence score was less predictive (HR 1.48 [95% CI 1.22–1.78]; LR- $\Delta\chi^2 = 13.68$ ;  $p = 0.0002$ ) and IHC4 was similar (HR 1.69 [95% CI 1.51–2.56]; LR- $\Delta\chi^2 = 22.83$ ;  $p < 0.0001$ ). All further analyses were done with the BCI-L model. In a multivariable analysis, all assays had significant prognostic ability for early distant recurrence (BCI-L HR 2.77 [95% CI 1.63–4.70], LR- $\Delta\chi^2 = 15.42$ ,  $p < 0.0001$ ; 21-gene recurrence score HR 1.80 [1.42–2.29], LR- $\Delta\chi^2 = 18.48$ ,  $p < 0.0001$ ; IHC4 HR 2.90 [2.01–4.18], LR- $\Delta\chi^2 = 29.14$ ,  $p < 0.0001$ ); however, only BCI-L was significant for late distant recurrence (BCI-L HR 1.95 [95% CI 1.22–3.14], LR- $\Delta\chi^2 = 7.97$ ,  $p = 0.0048$ ; 21-gene recurrence score HR 1.13 [0.82–1.56], LR- $\Delta\chi^2 = 0.48$ ,  $p = 0.47$ ; IHC4 HR 1.30 [0.88–1.94], LR- $\Delta\chi^2 = 1.59$ ,  $p = 0.20$ ).

The significance of our findings are that this was the first published study to provide a comparative multibiomarker analysis of early and late disease recurrence in a large randomized clinical trial of adjuvant hormonal therapy in postmenopausal patients with estrogen-receptor-positive breast cancer. Our results show that the breast-cancer index assay (BCI) is prognostic for both early and late distant recurrences in patients with



estrogen-receptor-positive breast cancer and identifies two distinct, clinically actionable populations of patients: those who are at low risk of recurrence and who might be adequately treated with adjuvant hormonal therapy alone, and those who are susceptible to a late recurrence and could be considered for extended adjuvant hormonal therapy or alternative therapy. Clinically, BCI could allow many women with early-stage oestrogen-receptor-positive breast cancer to avoid unnecessary extended antihormonal treatment, and could be an important method to aid the management of residual risk after 5 years of adjuvant hormonal treatment.

### **Publications:**

Zhang Y, Schnabel CA, Schroeder BE, Jerevall PL, Jankowitz RC, Fornander T, Stal O, Brufsky AM, **Sgroi D**, Erlander M. Breast Cancer Index Identifies Early Stage ER+ Breast Cancer Patients at Risk for Early and Late Distant Recurrence. Clin Cancer Res. 2013 Aug 1;19(15):4196-205.

**Sgroi, DC**, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, Erlander MG, Dunbier A, Sidhu K, Lopez-Knowles E, Goss PE, and Dowsett M. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the Breast Cancer Index (BCI) assay, 21-gene recurrence score, and IHC4 in TransATAC study population. Lancet Oncol. 2013 Oct;14(11):1067-76 (see PDF of publication below).

### **Abstracts:**

Sgroi, DC., Sestak, I., Zhang Y, Erlander, MG., Schnabel, CA., Goss, PE., Cuzick J, Dowsett M. Comparative Performance of Breast Cancer Index (BCI) vs. Oncotype Dx and IHC4 in the Prediction of Late Recurrence in Hormonal Receptor-Positive Lymph Node-Negative Breast Cancer Patients: A TransATAC Study. 2012 San Antonio Breast Cancer Symposium Abstract 3042.

Sgroi DC, Sestak I, Zhang Y, Erlander MG, Schnabel CA, Goss PE, Cuzick J, Dowsett M. Evaluation of Prognostic and Predictive Performance of Breast Cancer Index and Its Components in Hormonal Receptor-Positive Breast Cancer Patients: A TransATAC Study. 2012 San Antonio Breast Cancer Symposium Abstract 1176.



# Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population

Dennis C Sgroi, Ivana Sestak, Jack Cuzick, Yi Zhang, Catherine A Schnabel, Brock Schroeder, Mark G Erlander, Anita Dunbier, Kally Sidhu, Elena Lopez-Knowles, Paul E Goss, Mitch Dowsett

## Summary

**Background** Biomarkers to improve the risk–benefit of extended adjuvant endocrine therapy for late recurrence in patients with oestrogen-receptor-positive breast cancer would be clinically valuable. We compared the prognostic ability of the breast-cancer index (BCI) assay, 21-gene recurrence score (Oncotype DX), and an immunohistochemical prognostic model (IHC4) for both early and late recurrence in patients with oestrogen-receptor-positive, node-negative (N0) disease who took part in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) clinical trial.

**Methods** In this prospective comparison study, we obtained archival tumour blocks from the TransATAC tissue bank from all postmenopausal patients with oestrogen-receptor-positive breast cancer from whom the 21-gene recurrence score and IHC4 values had already been derived. We did BCI analysis in matched samples with sufficient residual RNA using two BCI models—cubic (BCI-C) and linear (BCI-L)—using previously validated cutoffs. We assessed prognostic ability of BCI for distant recurrence over 10 years (the primary endpoint) and compared it with that of the 21-gene recurrence score and IHC4. We also tested the ability of the assays to predict early (0–5 years) and late (5–10 years) distant recurrence. To assess the ability of the biomarkers to predict recurrence beyond standard clinicopathological variables, we calculated the change in the likelihood-ratio  $\chi^2$  (LR- $\Delta\chi^2$ ) from Cox proportional hazards models.

**Findings** Suitable tissue was available from 665 patients with oestrogen-receptor-positive, N0 breast cancer for BCI analysis. The primary analysis showed significant differences in risk of distant recurrence over 10 years in the categorical BCI-C risk groups ( $p < 0.0001$ ) with 6.8% (95% CI 4.4–10.0) of patients in the low-risk group, 17.3% (12.0–24.7) in the intermediate group, and 22.2% (15.3–31.5) in the high-risk group having distant recurrence. The secondary analysis showed that BCI-L was a much stronger predictor for overall (0–10 year) distant recurrence compared with BCI-C (interquartile HR 2.30 [95% CI 1.62–3.27]; LR- $\Delta\chi^2 = 22.69$ ;  $p < 0.0001$ ). When compared with BCI-L, the 21-gene recurrence score was less predictive (HR 1.48 [95% CI 1.22–1.78]; LR- $\Delta\chi^2 = 13.68$ ;  $p = 0.0002$ ) and IHC4 was similar (HR 1.69 [95% CI 1.51–2.56]; LR- $\Delta\chi^2 = 22.83$ ;  $p < 0.0001$ ). All further analyses were done with the BCI-L model. In a multivariable analysis, all assays had significant prognostic ability for early distant recurrence (BCI-L HR 2.77 [95% CI 1.63–4.70], LR- $\Delta\chi^2 = 15.42$ ,  $p < 0.0001$ ; 21-gene recurrence score HR 1.80 [1.42–2.29], LR- $\Delta\chi^2 = 18.48$ ,  $p < 0.0001$ ; IHC4 HR 2.90 [2.01–4.18], LR- $\Delta\chi^2 = 29.14$ ,  $p < 0.0001$ ); however, only BCI-L was significant for late distant recurrence (BCI-L HR 1.95 [95% CI 1.22–3.14], LR- $\Delta\chi^2 = 7.97$ ,  $p = 0.0048$ ; 21-gene recurrence score HR 1.13 [0.82–1.56], LR- $\Delta\chi^2 = 0.48$ ,  $p = 0.47$ ; IHC4 HR 1.30 [0.88–1.94], LR- $\Delta\chi^2 = 1.59$ ,  $p = 0.20$ ).

**Interpretation** BCI-L was the only significant prognostic test for risk of both early and late distant recurrence and identified two risk populations for each timeframe. It could help to identify patients at high risk for late distant recurrence who might benefit from extended endocrine or other therapy.

**Funding** Avon Foundation, National Institutes of Health, Breast Cancer Foundation, US Department of Defense Breast Cancer Research Program, Susan G Komen for the Cure, Breakthrough Breast Cancer through the Mary-Jean Mitchell Green Foundation, AstraZeneca, Cancer Research UK, and the National Institute for Health Research Biomedical Research Centre at the Royal Marsden (London, UK).

## Introduction

Oestrogen-receptor-positive breast cancer is a disease with a protracted risk of recurrence.<sup>1,2</sup> After 5 years of adjuvant tamoxifen, patients have a sustained risk of disease recurrence and death for at least 15 years after diagnosis.<sup>1</sup> Long-term follow-up from pivotal upfront

trials of adjuvant aromatase inhibitors, including the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial<sup>3,4</sup> and Breast International Group (BIG) 1-98 study,<sup>5</sup> show a continuing rate of recurrence of about 2% per year after initial therapy, with greater than half of all recurrences occurring after 5 years of

*Lancet Oncol* 2013; 14: 1067–76

Published Online  
September 12, 2013  
[http://dx.doi.org/10.1016/S1470-2045\(13\)70387-5](http://dx.doi.org/10.1016/S1470-2045(13)70387-5)

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adjuvant endocrine therapy.<sup>3–5</sup> These findings emphasise the need for extended adjuvant therapy and a biomarker that can guide the treatment decision-making process.

Multigene expression signatures studied in the past decade for assessment of recurrence risk in oestrogen-receptor-positive breast cancer rely mainly on the quantitative measurement of proliferation-related gene expression.<sup>6–15</sup> These multigene signatures, including the 21-gene recurrence score (Oncotype DX; Genomic Health, Redwood City, CA, USA), are strong predictors of distant recurrence, but their prognostic ability diminishes when assessing risk beyond 5 years from diagnosis.<sup>16,17</sup> By contrast, predictors of late recurrence are not well characterised, and different mechanisms might be associated with early and late recurrences.<sup>18,19</sup> An unmet clinical need exists for biomarkers that identify patients who are adequately treated with only 5 years of endocrine therapy, and conversely, those at increased risk of late

recurrence who might warrant extended adjuvant endocrine or other therapy.

Members of our study team (specifically, those from Massachusetts General Hospital, Boston, MA, USA, and bioTheranostics, San Diego, CA, USA) previously developed and validated the breast-cancer index (BCI) assay that consists of two independently developed gene expression biomarkers: molecular grade index (MGI) and *HOXB13/IL17BR*.<sup>20,21</sup> MGI, a five-gene predictor that recapitulates tumour grade and proliferation, is highly prognostic in patients with oestrogen-receptor-positive breast cancer.<sup>22</sup> *HOXB13/IL17BR*, which was developed independently of tumour grade or proliferation, is prognostic for early and late distant recurrences, and is predictive of extended adjuvant aromatase inhibitor benefit in patients with early-stage oestrogen-receptor-positive breast cancer.<sup>12,23,24</sup> Both the BCI and 21-gene recurrence score assays measure gene expression by quantitative real-time PCR, although they differ in the genes that they detect.<sup>6,22</sup> IHC4, developed by members of our study team (MD and JC), is another prognostic model that measures protein expression of four of the most informative immunohistochemical biomarkers: oestrogen receptors, progesterone receptors, HER2, and Ki-67,<sup>25</sup> none of which are encoded by genes in the BCI assay. BCI has not been assessed in patients with oestrogen-receptor-negative or triple-negative breast cancer.

In this study, our objective was to assess the prognostic value of BCI for early and late distant recurrence in postmenopausal women with localised lymph-node-negative (N0) breast cancer given either tamoxifen or anastrozole monotherapy in the ATAC trial, and to compare its prognostic ability in matched patients with that of the 21-gene recurrence score and IHC4.

## Methods

### Study design and patients

In this prospective comparison study, we obtained tissue samples from the TransATAC project, initiated in 2002 to establish a tissue bank of formalin-fixed paraffin-embedded (FFPE) primary tumour blocks from postmenopausal patients with oestrogen-receptor-positive breast cancer from the monotherapy groups of the ATAC trial to assist with translational research.<sup>3,4</sup> We requested archival tumour blocks from all patients for whom the 21-gene recurrence score and IHC4 had already been calculated,<sup>25,26</sup> except those known to be oestrogen-receptor and progesterone-receptor negative according to local tests and those randomly assigned to the combination treatment group of the ATAC trial. This study was approved by the South-East London Research Ethics Committee and the Massachusetts General Hospital Institutional Review Board. Patients had provided written consent for their tissue to be used in further trials.

|                           | Comparison between TransATAC and non-TransATAC patients |                                   | p value† | HER2-negative, N0, BCI TransATAC subgroup (n=597) |
|---------------------------|---|-----------------------------------|----------|---|
|                           | N0, BCI TransATAC cohort (n=665)                        | N0, non-TransATAC cohort* (n=561) |          |   |
| Age (years)               | 62.4 (46.7–88.4)  | 62.3 (46.0–92.8)                  | 0.12     | 62.7 (46.7–85.1)                                  |
| BMI (kg/m <sup>2</sup> )  | 27.1 (4.8)  | 26.8 (5.1)                        | 0.28     | 27.2 (4.8)  |
| Tumour size               |   |                                   | 0.13     |   |
| <2 cm                     | 486 (73%)   | 432 (77%)                         | ..       | 442 (74%)   |
| 2–3 cm                    | 144 (22%)   | 95 (17%)                          | ..       | 125 (21%)   |
| >3 cm                     | 35 (5%)   | 29 (5%)                           | ..       | 30 (5%)   |
| Unknown                   | 0   | 5 (1%)                            | ..       | 0   |
| Tumour grade              |   |                                   | 0.0051   |   |
| Well differentiated       | 143 (22%)   | 155 (28%)                         | ..       | 138 (23%)   |
| Moderately differentiated | 395 (59%)   | 300 (53%)                         | ..       | 357 (60%)   |
| Poorly differentiated     | 127 (19%)   | 78 (14%)                          | ..       | 102 (17%)   |
| Unknown                   | 0   | 28 (5%)                           | ..       | 0   |
| Radiotherapy              |   |                                   | 0.95     |   |
| No                        | 220 (33%)   | 187 (33%)                         | ..       | 189 (32%)   |
| Yes                       | 445 (67%)   | 374 (67%)                         | ..       | 408 (68%)   |
| Mastectomy                |   |                                   | 0.86     |   |
| No                        | 439 (66%)   | 374 (67%)                         | ..       | 404 (68%)   |
| Yes                       | 226 (34%)   | 187 (33%)                         | ..       | 193 (32%)   |
| Treatment                 |   |                                   | 0.95     |   |
| Anastrozole               | 337 (51%)   | 285 (51%)                         | ..       | 309 (52%)   |
| Tamoxifen                 | 328 (49%)   | 276 (49%)                         | ..       | 288 (48%)   |
| Distant recurrence        |   |                                   |          |   |
| Early (0–5 years)         | 33 (5%)   | 23 (4%)                           | 0.56     | 21 (4%)   |
| Late (5–10 years)         | 39 (6%)   | 12 (2%)                           | 0.0022   | 36 (7%)   |

Data are median (range), mean (SD), or number (%), unless otherwise specified. All patients had oestrogen-receptor-positive, N0 breast cancer. TransATAC=translational arm of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. N0=node negative. BCI=breast-cancer index assay. BMI=body-mass index. \*Patients who participated in the single-agent groups of the ATAC trial, but for whom tumour blocks were not available for TransATAC. †t tests were used for age and BMI, a proportional test based on normal approximation was used for distant recurrence, and a Fisher's exact test was used for all other comparisons.

**Table 1: Patient demographic and clinical characteristics**

## Procedures

Previously, a study was done in which RNA was extracted from FFPE blocks from the TranATAC tissue bank from UK patients (whose samples made up 79% of the collection) to calculate and test the 21-gene recurrence score.<sup>26</sup> Subsequently, immunohistochemical analysis for oestrogen receptors, progesterone receptors, HER2, and Ki-67 and tumour grade assessment were undertaken,<sup>25</sup> and IHC4 and clinical treatment score (a prognostic model using the classic variables of tumour size and grade, lymph node status, age, and treatment) were calculated using tissue samples from the same patients whose tissue was used to calculate the 21-gene recurrence score (for whom sufficient additional tissue was available).

In our study, members of our team at bioTheranostics (YZ, CAS, BS, and MGE), who were masked to clinical outcome, used the same matched samples as used in the previous studies<sup>25,26</sup> with sufficient residual RNA to undertake BCI analysis. We tested the genes, analysed the primer and probe sequences, undertook the RT-PCR procedures, and calculated *HOXB13/IL17BR* and MGI as previously described.<sup>20,22,23</sup> We used two prespecified BCI models—cubic (BCI-C) and linear (BCI-L), based on cubic and linear combinations of the variables—which had been previously developed and validated in the tamoxifen treatment group and the non-treatment group of the Stockholm trial,<sup>20,21,27</sup> respectively.

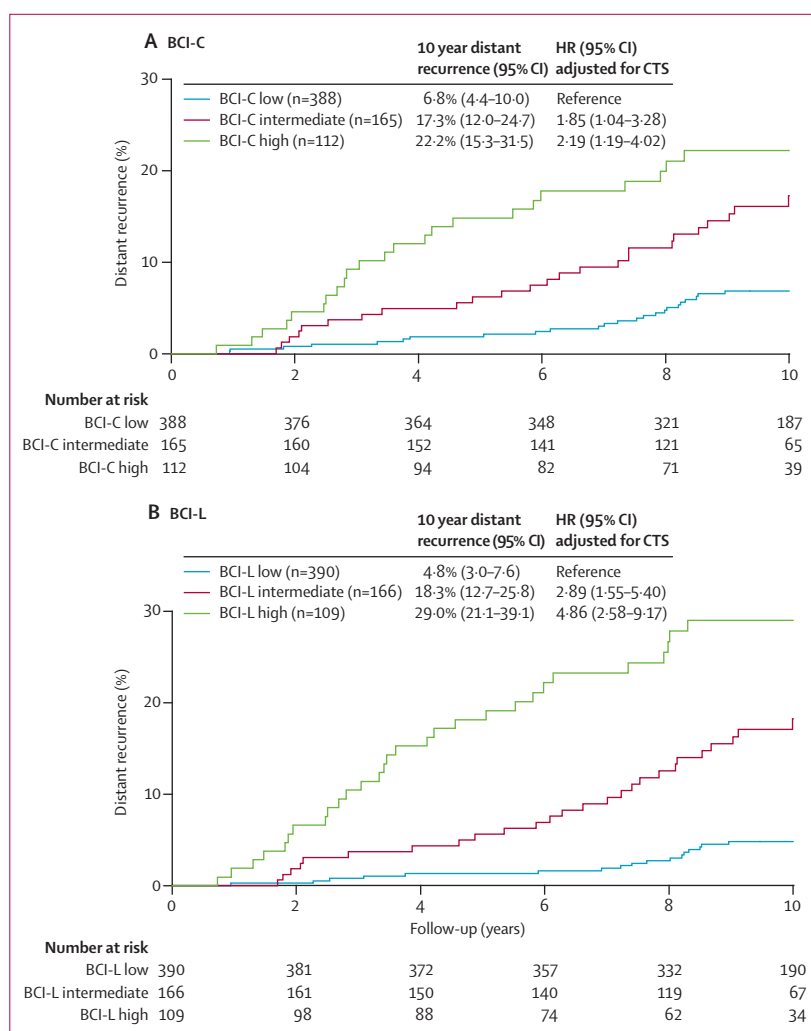
The BCI score was linearly scaled to a final score (0–10). We identified low-risk, intermediate-risk, and high-risk groups with prespecified cutoff points for each model: BCI-C low risk (<5.0 points), BCI-C intermediate risk (≥5.0–6.4), and BCI-C high risk (>6.4); and BCI-L low risk (<5.0825 points), BCI-L intermediate risk (≥5.0825–6.5025), and BCI-L high risk (>6.5025).<sup>21</sup> The 21-gene recurrence score risk groups were identified as previously described.<sup>5</sup> Three IHC4 risk groups were established using two cutoff points that corresponded to a 10 year distant recurrence rate of 10% and 20% (ie, <10%, ≥10–20%, and >20%) in the TransATAC cohort, respectively. The IHC4 cutoffs have not been independently validated.

We prospectively defined distant recurrence as the primary endpoint, which refers to all recurrences at distant organs, excluding contralateral disease, loco-regional and ipsilateral recurrences, and other second primary cancers; we included distant recurrences that took place after locoregional recurrence as an event at the time of distant recurrence. We censored patients who died before distant recurrence. We also defined all recurrences, breast-cancer deaths, and overall survival (time to death from any cause) as secondary endpoints. The primary analysis population was patients with oestrogen-receptor-positive, N0 breast cancer, whereas the secondary analysis populations included patients with oestrogen-receptor-positive, N0, HER2-negative breast cancer and those with oestrogen-receptor-positive, node-positive breast cancer. We prospectively defined the primary study objective as assessment of overall

(0–10 year) prognostic ability of the BCI-C model for distant recurrence in patients with oestrogen-receptor-positive, N0 breast cancer. Secondary objectives were to assess the prognostic ability of the BCI-L model and its components, *HOXB13/IL17BR* and MGI, for overall (0–10 year), early (0–5 year), and late (5–10 year) distant recurrence, as well as to compare the ability of BCI-L with that of the recurrence score and IHC4.

## Statistical analysis

A statistical analysis plan was approved by the steering committee for the ATAC and LATTE (Long-term Anastrozole versus Tamoxifen Treatment Effects) trials<sup>3</sup> before study initiation. We assessed early distant recurrences by censoring follow-up of all patients 5 years after diagnosis. We assessed late distant recurrences within the subset of patients who remained



**Figure 1: Prognostic ability of BCI-C and BCI-L for overall 10-year distant recurrence**  
Prognostic value of prespecified risk groups according to BCI-C (A) and BCI-L (B) for overall 10-year distant recurrences in all patients with oestrogen-receptor-positive, node-negative breast cancer. BCI-C=breast-cancer index assay (cubic model). BCI-L=breast-cancer index assay (linear model). CTS=clinical treatment score. HR=hazard ratio.

distant-recurrence free for at least 5 years to assess whether the gene signature remained prognostic after its prognostic effect for early recurrence was removed. We used likelihood ratio tests based on Cox proportional hazards regression models to test for a significant difference between a reduced proportional hazards model based on clinical treatment score and a full proportional hazards model, including BCI, 21-gene recurrence score, or IHC4. We quantified the improvement in prediction by the change in the likelihood ratio  $\chi^2$  (LR- $\Delta\chi^2$ ) value, which measures the amount of information added to the proportional hazards model by the gene signatures compared with clinical treatment score. Because IHC4 was developed in a subset of TransATAC samples, sample splitting was done, as previously described,<sup>25</sup> to adjust for potential overfitting. We used Kaplan-Meier survival analysis to graphically present the proportion of patients with distant recurrence in BCI's three prespecified risk groups, and tested the equality of the curves with a log-rank test.

We calculated the risk of distant recurrence as a function of BCI as a linear covariate from Cox proportional hazards models for overall (0–10 years), early (0–5 years), and late (5–10 years) distant recurrence. To compare the ability of BCI, 21-gene recurrence score, and IHC4, we estimated the interquartile hazard ratio (HR), comparing the 75th percentile versus the 25th percentile of the continuous scores of these biomarkers and the associated 95% CI from

Cox proportional hazards models. We regarded a two-sided p value of less than 0.05 to be statistically significant. Because the recurrence score had already been studied in TransATAC, and IHC4 was developed in a subset of these patients,<sup>25,26</sup> the ability of the 21-gene recurrence score and IHC4 as continuous scores was prespecified, and we did not plan or do any multiple testing adjustment. We did statistical analyses with STATA version 12.1.

### Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors have not been paid to write this article by a pharmaceutical company or other agency. The BCI assays were undertaken at bioTheranostics by laboratory personnel (YZ, CAS, BS, and MGE) who had no knowledge of treatment assignment or clinical outcome. The study biostatisticians (IS, JC) were the only people to have direct access to the clinical outcome raw data and the raw data for the 21-gene recurrence score and IHC4. The corresponding author had full access to all BCI assay raw data in the study and had final responsibility for the decision to submit for publication.

### Results

Values for 21-gene recurrence score, IHC4, and BCI were calculated for 915 women, of whom 665 had

|  | All recurrence (0–10 years) |                              | Early recurrence (0–5 years) |                              | Late recurrence (5–10 years) |                              |
|--|-----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|  | HR* (95% CI)                | LR- $\Delta\chi^2$ (p value) | HR* (95% CI)                 | LR- $\Delta\chi^2$ (p value) | HR* (95% CI)                 | LR- $\Delta\chi^2$ (p value) |
| <b>Univariate</b>                                      |                             |                              |                              |                              |                              |                              |
| <b>BCI</b>   |                             |                              |                              |                              |                              |                              |
| N0   | 3.12 (2.25–4.32)            | 49.07 (p<0.0001)             | 4.11 (2.52–6.70)             | 34.58 (p<0.0001)             | 2.47 (1.59–3.83)             | 17.37 (p<0.0001)             |
| N0 HER2-negative                                       | 3.30 (2.30–4.73)            | 46.03 (p<0.0001)             | 4.22 (2.32–7.64)             | 25.86 (p<0.0001)             | 2.84 (1.80–4.48)             | 22.66 (p<0.0001)             |
| <b>21-gene recurrence score</b>                        |                             |                              |                              |                              |                              |                              |
| N0   | 1.64 (1.39–1.94)            | 27.37 (p<0.0001)             | 1.96 (1.60–2.41)             | 28.09 (p<0.0001)             | 1.28 (0.95–1.72)             | 2.99 (p=0.21)                |
| N0 HER2-negative                                       | 1.89 (1.45–2.47)            | 19.55 (p<0.0001)             | 2.38 (1.61–3.53)             | 16.18 (p<0.0001)             | 1.59 (1.09–2.31)             | 6.65 (p=0.014)               |
| <b>IHC4</b>  |                             |                              |                              |                              |                              |                              |
| N0   | 2.30 (1.80–2.95)            | 40.90 (p<0.0001)             | 3.38 (2.39–4.78)             | 42.46 (p<0.0001)             | 1.55 (1.06–2.26)             | 5.58 (p=0.022)               |
| N0 HER2-negative                                       | 2.66 (1.85–3.81)            | 27.04 (p<0.0001)             | 4.08 (2.26–7.36)             | 22.13 (p<0.0001)             | 2.06 (1.29–3.28)             | 9.32 (p=0.0034)              |
| <b>Multivariate including clinical treatment score</b> |                             |                              |                              |                              |                              |                              |
| <b>BCI</b>   |                             |                              |                              |                              |                              |                              |
| N0   | 2.30 (1.62–3.27)            | 22.69 (p<0.0001)             | 2.77 (1.63–4.70)             | 15.42 (p<0.0001)             | 1.95 (1.22–3.14)             | 7.97 (p=0.0048)              |
| N0 HER2-negative                                       | 2.49 (1.68–3.68)            | 21.99 (p<0.0001)             | 3.26 (1.69–6.30)             | 13.65 (p=0.00023)            | 2.12 (1.30–3.47)             | 9.453 (p=0.0021)             |
| <b>21-gene recurrence score</b>                        |                             |                              |                              |                              |                              |                              |
| N0   | 1.48 (1.22–1.78)            | 13.68 (p=0.00020)            | 1.80 (1.42–2.29)             | 18.48 (p<0.0001)             | 1.13 (0.82–1.56)             | 0.48 (p=0.47)                |
| N0 HER2-negative                                       | 1.52 (1.15–2.02)            | 7.65 (p=0.0055)              | 1.93 (1.26–2.96)             | 8.37 (p=0.0041)              | 1.28 (0.87–1.88)             | 1.33 (p=0.28)                |
| <b>IHC4</b>  |                             |                              |                              |                              |                              |                              |
| N0   | 1.69 (1.51–2.56)            | 22.83 (p<0.0001)             | 2.90 (2.01–4.18)             | 29.14 (p<0.0001)             | 1.30 (0.88–1.94)             | 1.59 (p=0.20)                |
| N0 HER2-negative                                       | 2.13 (1.45–3.14)            | 13.75 (p=0.00019)            | 3.41 (1.83–6.39)             | 13.83 (p<0.0001)             | 1.61 (0.98–2.66)             | 3.30 (p=0.086)               |

HR=hazard ratio. LR- $\Delta\chi^2$ =change in the  $\chi^2$  value based on the likelihood ratio statistic. BCI=breast-cancer index assay. N0=node negative. IHC4=four immunohistochemical markers (oestrogen receptor, progesterone receptor, HER2, and Ki-67). \*HR was calculated as between the IQR of the continuous scores of each biomarker; sample splitting was used to calculate HRs and  $\chi^2$  for IHC4.

**Table 2: Comparative prognostic ability for overall, early, and late distant recurrence of BCI, 21-gene recurrence score, and IHC4 in all patients and in the HER2-negative subset**



oestrogen-receptor-positive, N0 breast cancer (appendix p 6). Clinical characteristics of these 665 patients are listed in table 1 and compared with the characteristics of 561 UK patients with oestrogen-receptor-positive, N0 breast cancer who participated in the ATAC trial but who were not part of TransATAC. We noted no significant differences between these two groups, except that the non-TransATAC cohort had significantly more well differentiated tumours and less moderately differentiated tumours than the TransATAC patients, and significantly fewer late distant recurrences. In N0 women in the BCI TransATAC cohort, there were 106 recurrences, including 72 distant recurrences and seven local recurrences after mastectomy. Median follow-up in the BCI TransATAC cohort was 9.97 years (IQR 8.5–10).

In the 665 oestrogen-receptor-positive, N0 patients, Kaplan-Meier analysis of the BCI-C model showed significant differences in absolute distant recurrence over a 10 year period ( $p < 0.0001$ ) in the prespecified categorical BCI-C risk groups, and differences in the HRs between the low-risk group and the other risk groups, after adjustment for the effects of tumour size and grade, age, and treatment (as determined by clinical treatment score; figure 1A). BCI-C analysed as a continuous variable—rather than as subgroups with defined cutoffs—was not significantly associated with overall (0–10 year) risk of distant recurrence when adjusted for clinical treatment score (interquartile HR 1.39 [95% CI 0.99–3.70];  $LR-\Delta\chi^2=3.70$ ;  $p=0.054$ ).

Assessment of BCI-L in the same population of patients showed that this version was much more strongly associated with overall risk of distant recurrence than was BCI-C when adjusted for clinical treatment score (interquartile HR 2.30 [95% CI 1.62–3.27];  $LR-\Delta\chi^2=22.69$ ;  $p < 0.0001$ ; table 2). Kaplan-Meier curves show clear differences in absolute distant recurrence rates according to prespecified BCI-L risk groups ( $p < 0.0001$ ; figure 1B). The overall 10-year risk of distant recurrence increased linearly with increasing BCI-L (appendix p 7).

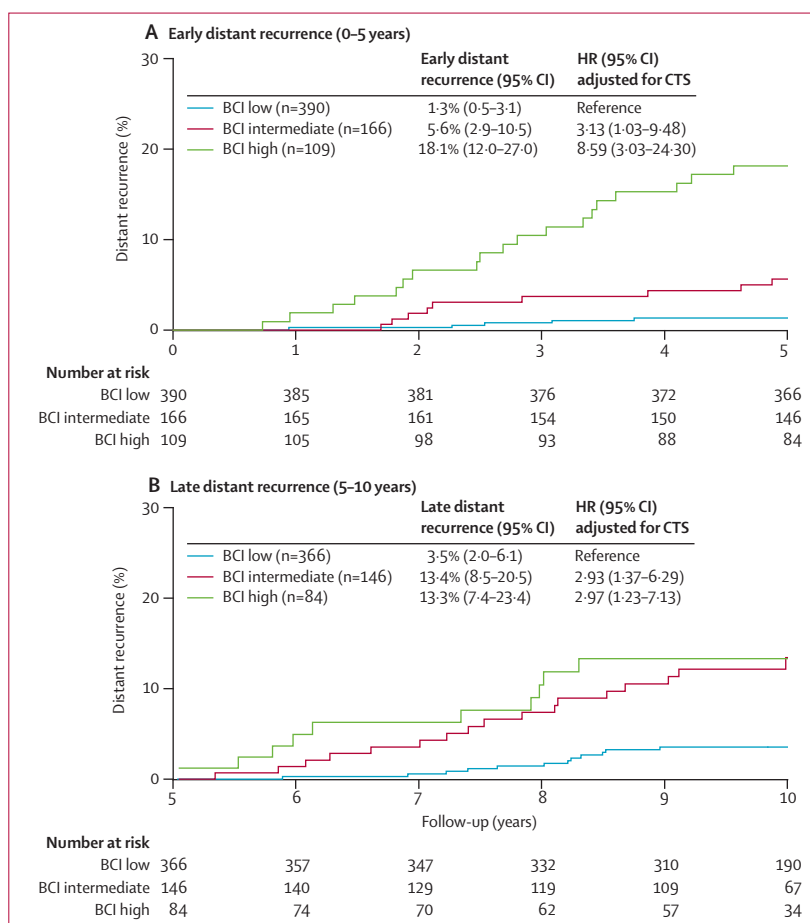
In the HER2-negative, N0 subset of 597 patients, both BCI-C and BCI-L were significantly associated with overall risk of distant recurrence (BCI-C, interquartile HR 1.65 [95% CI 1.12–2.43],  $LR-\Delta\chi^2=6.61$ ,  $p=0.0101$ ; BCI-L, interquartile HR 2.49 [1.68–3.68],  $LR-\Delta\chi^2=21.9$ ,  $p < 0.0001$ ; table 2). Kaplan-Meier curves of the prespecified risk groups for both versions of BCI showed distinct differences in absolute distant recurrence (appendix p 8).

Comparison of the prognostic ability of BCI-L with that of BCI-C showed that, unlike BCI-C, BCI-L was a significant predictor of risk of recurrence as both a continuous and categorical variable, and the HR, after adjustment for clinical treatment score, was 2.19 versus 4.86 between high-risk and low-risk groups for BCI-C and BCI-L, respectively. Thus, we did all subsequent analyses using the linear model (henceforth referred to as BCI).

BCI was significantly associated with risk of early (0–5 year) distant recurrence (table 2) when adjusted for clinical treatment score. Kaplan-Meier curves (figure 2A) displayed differences in absolute distant recurrence rates at 5 years. Although three risk groups were prespecified, the results from the prespecified Kaplan-Meier analysis showed low-risk and intermediate-risk patients had similar rates of distant recurrence and constitute one group that is distinctly different from the group of high-risk patients. A post-hoc Kaplan-Meier analysis showed little difference in distant recurrence at 5 years between the BCI low-risk and intermediate-risk groups, which contained 556 (84%) of 665 patients (P1) with a combined 5 year rate of distant recurrence of 2.6% (95% CI 1.5–4.3; appendix p 1). The BCI high-risk group (P2) that contained 109 (16%) of 665 patients had a 5 year rate of distant recurrence of 18.1% (95% CI 12.0–27.0). When adjusted for clinical treatment score, the HR between P1 and P2 was 4.61 (95% CI 2.20–9.66).

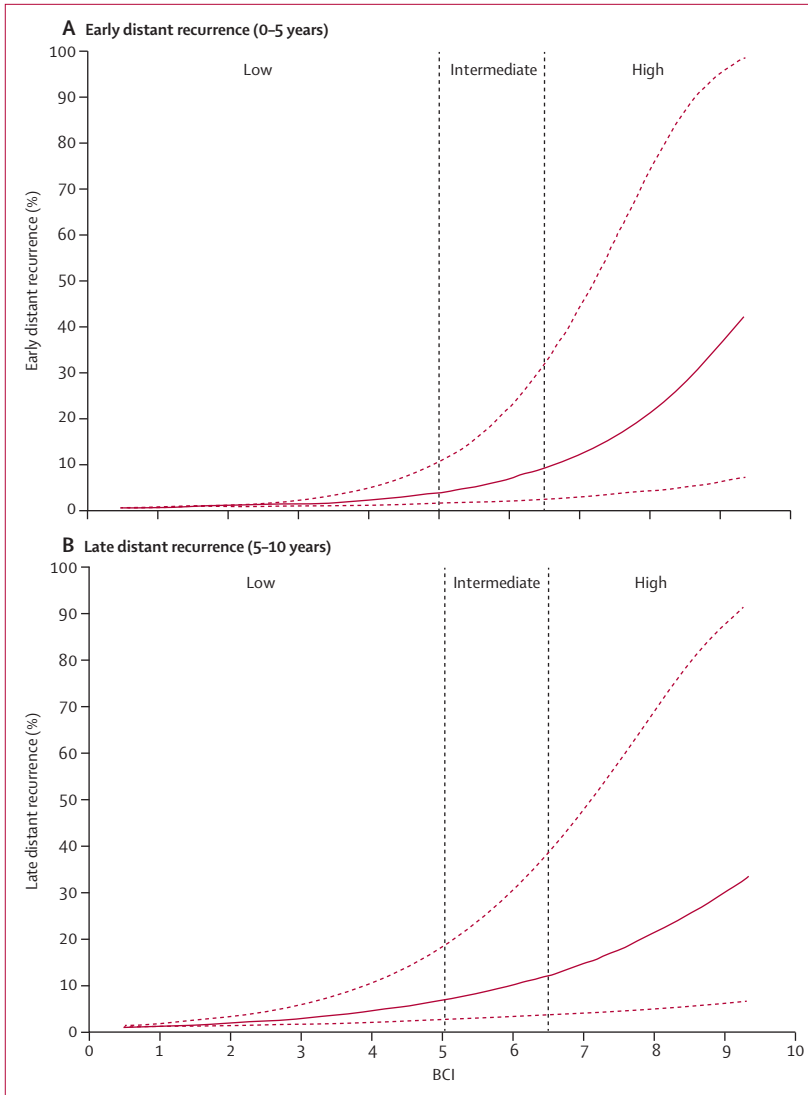
For late (5–10 year) recurrence, BCI was significantly associated with risk of distant recurrence (table 2) when

See Online for appendix



**Figure 2: BCI prognostic ability for overall early and late distant recurrence**

Prognostic value of BCI prespecified risk groups for early, 0–5 years (A), and late, 5–10 years (B), distant recurrences in patients with oestrogen-receptor-positive, node-negative breast cancer. BCI=breast-cancer index assay (linear model). CTS=clinical treatment score. HR=hazard ratio.



**Figure 3: Early and late distant recurrence as a function of continuous BCI values**  
Risk of early, 0–5 years (A), and late, 5–10 years (B), distant recurrence as a function of continuous BCI score in patients with oestrogen-receptor-positive, node-negative breast cancer. Dotted vertical lines delineate the borders between the low, intermediate, and high prespecified BCI risk groups. The dotted curves show 95% CI. BCI=breast-cancer index assay (linear model).

|  | Early recurrence (0–5 years) |                              | Late recurrence (5–10 years) |                              |
|--|------------------------------|------------------------------|------------------------------|------------------------------|
|  | HR* (95% CI)                 | LR- $\Delta\chi^2$ (p value) | HR* (95% CI)                 | LR- $\Delta\chi^2$ (p value) |
| <b>Univariate</b>                                      |                              |                              |                              |                              |
| MGI  | 3.15 (1.96–5.06)             | 24.27 (p<0.0001)             | 1.74 (1.16–2.62)             | 7.26 (p=0.0070)              |
| HOXB13/IL17BR  | 2.42 (1.44–4.07)             | 10.90 (p=0.0010)             | 2.25 (1.38–3.66)             | 10.32 (p=0.0013)             |
| <b>Multivariate including clinical treatment score</b> |                              |                              |                              |                              |
| MGI  | 2.10 (1.25–3.52)             | 8.41 (p=0.0037)              | 1.33 (0.86–2.06)             | 1.70 (p=0.19)                |
| HOXB13/IL17BR  | 2.03 (1.20–3.41)             | 7.05 (p=0.0079)              | 2.02 (1.25–3.26)             | 8.17 (p=0.0043)              |

HR=hazard ratio. LR- $\Delta\chi^2$ =change in the  $\chi^2$  value based on the likelihood ratio statistic. MGI=molecular grade index. \*HR was calculated as between the IQR of MGI and HOXB13/IL17BR.

**Table 3: Prognostic ability of HOXB13/IL17BR and MGI for early and late distant recurrence in oestrogen-receptor-positive, node-negative patients**

adjusted for clinical treatment score. Kaplan-Meier curves showed differences in absolute distant recurrence rates for years 5–10 for the BCI low-risk, intermediate-risk, and high-risk groups (figure 2B). The results from the prespecified Kaplan-Meier analysis showed that intermediate-risk and high-risk patients had highly similar rates of recurrence, constituting one population that was distinctly different from the population of low-risk patients. Additional post-hoc Kaplan-Meier analyses showed that the distant recurrence of the BCI low-risk group (P3) that contained 366 (61%) of 596 patients, with a distant recurrence rate of 3.5% for years 5–10, was substantially different from that of combined BCI intermediate-risk and high-risk groups (P4), which constituted 230 (39%) of 596 patients and had a combined rate (years 5–10) of distant recurrence of 13.4% (95% CI 9.3–19.0; appendix p 1). Adjusting for clinical treatment score, the HR between P3 and P4 was 2.94 (95% CI 1.44–6.01). The risk of distant recurrence increased linearly with increasing BCI values for both early and late recurrence (figure 3).

Since the natural history of oestrogen-receptor-positive, HER2-positive breast cancer differs from that of oestrogen-receptor-positive HER2-negative breast cancer, we did a subset analysis to assess whether the prognostic ability of BCI in the entire N0 oestrogen-receptor-positive TransATAC cohort was unduly affected by the inclusion of the subset of HER2-positive patients. In the HER2-negative N0 subset of 597 patients (90% of the total tested study group), BCI was significantly associated with risk of early distant recurrence and late distant recurrence (table 2) and associated with distinct differences in absolute distant recurrence according to BCI risk groups (appendix p 9). For both early and late recurrence the risk of distant recurrence increased with increasing BCI values (appendix p 10).

For early recurrence, both the BCI components MGI and HOXB13/IL17BR added statistically significant prognostic information (table 3). However, for late recurrence, only HOXB13/IL17BR provided additional information beyond standard clinicopathological factors (table 3).

The correlation matrix of BCI-C, BCI-L, 21-gene recurrence score, IHC4, and clinical treatment score showed a strong correlation between the two versions of BCI model and between IHC4 and the 21-gene recurrence score, whereas both BCI-C and BCI-L had weak to moderate correlation (<0.5) with the 21-gene recurrence score, IHC4, and clinical treatment score (appendix p 2).

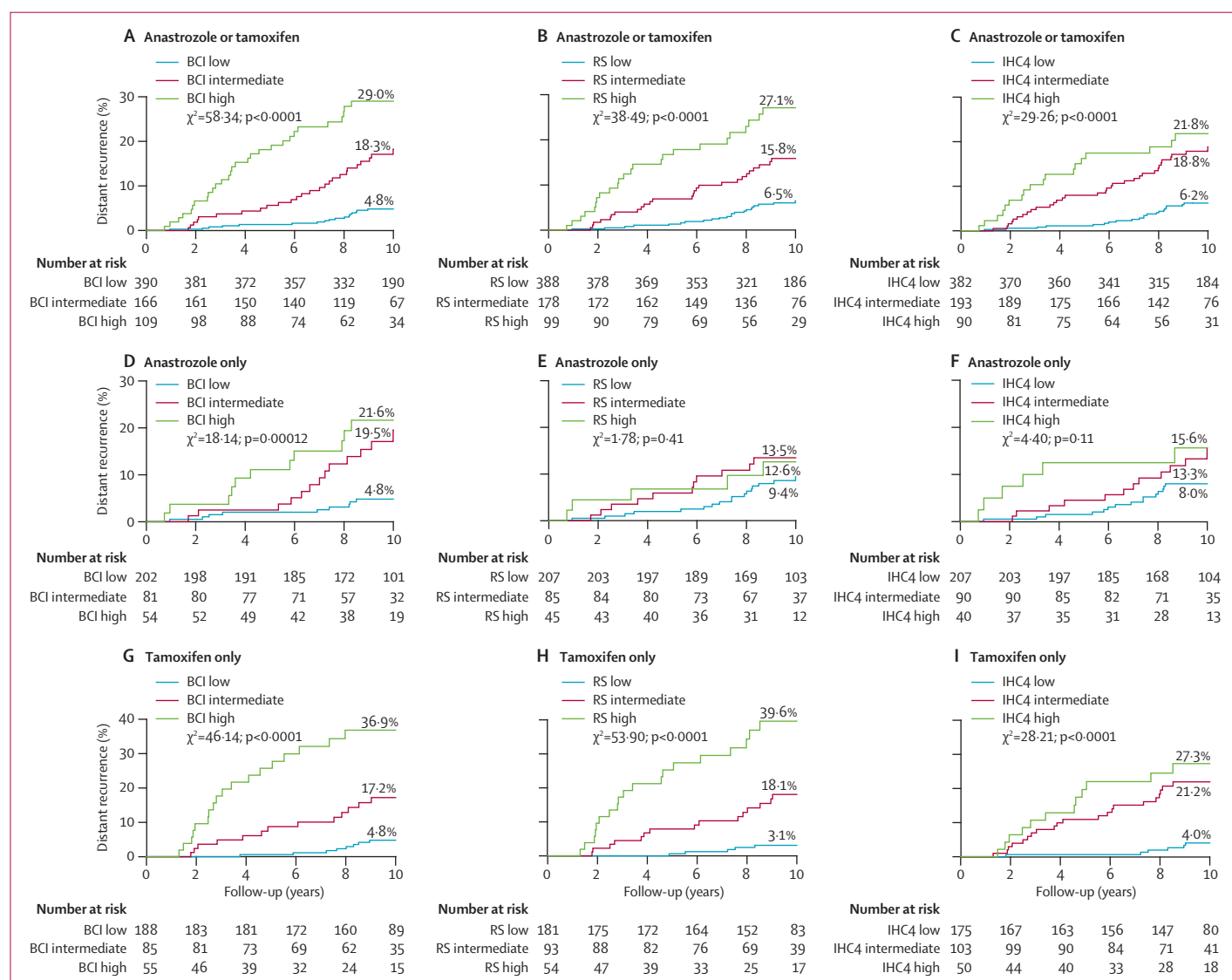
Kaplan-Meier curves of overall (0–10 year) distant recurrence for 21-gene recurrence score and IHC4 risk groups for all patients, and separately according to treatment group (anastrozole or tamoxifen), are shown in figure 4. For all patients combined (ie, those who received either anastrozole or tamoxifen), the BCI low-risk group had the lowest proportion of patients with distant recurrence in 10 years (4.8%, 95% CI 3.0–7.6) when compared with the 21-gene recurrence score



low-risk group (6.5%, 4.3–9.7) and the IHC4 low-risk group (6.2%, 4.1–9.3), whereas the BCI high-risk group had the highest proportion of distant recurrence (29.0%, 21.1–39.1) compared with the 21-gene recurrence score high-risk group (27.1%, 18.9–37.8) and IHC4 high-risk group (21.8%, 14.3–32.4; figure 4).

We used the change in likelihood ratio  $LR\Delta\chi^2$  values to provide a direct head-to-head comparison of BCI with IHC4 and 21-gene recurrence score. The relative prognostic ability of each biomarker varied depending on the distant recurrence timeframe (table 2). For early recurrence, BCI, IHC4, and the 21-gene recurrence score

were all prognostic for distant recurrence in both univariate and multivariate analyses (table 2). In all N0 patients, IHC4 was more prognostic than recurrence score and BCI after adjusting for clinical treatment score. However, in the N0 HER2-negative patients, BCI and IHC4 had similar prognostic abilities that were both better than that of the 21-gene recurrence score after adjusting for clinical treatment score (table 2). In the multivariate analysis of late recurrence, only BCI remained strongly prognostic in all N0 and N0 HER2-negative patients, whereas both IHC4 and 21-gene recurrence score were not prognostic in either population



**Figure 4: Prognostic ability of BCI, 21-gene recurrence score, and IHC4 for overall 10 year distant recurrence, for all patients and according to ATAC treatment group**

Prognostic value of the prespecified risk groups of BCI and 21-gene recurrence score and the post-hoc-determined categorical risk groups of IHC4 for overall 10 year distant recurrence in patients with oestrogen-receptor-positive, node-negative breast cancer. Graphs presented for all patients assigned to either anastrozole or tamoxifen for BCI (A), 21-gene recurrence score (B), and IHC4 (C); for patients assigned to anastrozole in the ATAC trial for BCI (D), 21-gene recurrence score (E), and IHC4 (F); and for patients assigned to tamoxifen in the ATAC trial for BCI (G), 21-gene recurrence score (H), and IHC4 (I). ATAC=Arimidex, Tamoxifen, Alone or in Combination. BCI=breast-cancer index assay (linear model). IHC4=four immunohistochemical markers (oestrogen receptor, progesterone receptor, HER 2, and Ki-67). RS=21-gene recurrence score.

(table 2). We noted similar results considering all recurrences, breast-cancer deaths, and overall survival as endpoints (appendix p 3).

Although the primary analysis of this study centred on N0 patients, an analysis of node-positive patients showed that BCI was also prognostic for distant recurrence in these patients (log rank  $p=0.0045$ ; appendix p 11). Furthermore, a comparative analysis showed that BCI, IHC4, and the 21-gene recurrence score had highly similar prognostic ability in this population of patients, albeit less robust than that noted in the N0 subset (appendix p 4).

## Discussion

We have shown that BCI (linear model) has a statistically significant prognostic ability over 10 years for the prediction of individual risk of distant recurrence in patients with oestrogen-receptor-positive, N0 breast cancer from the TransATAC cohort. Examining clinically relevant time periods of 0–5 years and 5–10 years separately showed that BCI might have the potential to affect two important decision points in the management of these patients. At baseline, BCI identified two apparently distinct groups of patients: a relatively small population (109 [16%] of 665) at high risk for early recurrence who do not benefit adequately from endocrine therapy alone and should be considered for additional therapy (eg, chemotherapy or some other treatment), and a large population (556 [84%] of 665) whose risk for early recurrence was sufficiently low that they might be regarded as adequately treated with endocrine therapy alone. For women who are disease free after 5 years of therapy with either upfront adjuvant tamoxifen or upfront aromatase inhibitor—the two most common adjuvant therapies in clinical use—BCI also identified two distinct groups: a group of patients (230 [39%] of 596) at substantial risk of late recurrence, and a second group (366 [61%] of 596) at very low risk of late recurrence (appendix p 1). For those at low risk after either upfront tamoxifen or aromatase inhibitor, BCI affords the option of no further systemic therapy of any sort. Members of our study team have recently shown in another study<sup>24</sup> that patients at high risk of recurrence after upfront adjuvant tamoxifen (patients with high *HOXB13/IL17BR*) benefit from extended hormonal therapy with the aromatase inhibitor letrozole.<sup>24</sup> Patients at high risk of recurrence after 5 years of upfront aromatase inhibitor might or might not benefit from extended adjuvant hormonal therapy or indeed from any systemic therapy. The management approach for these patients will in part be guided by results from the National Cancer Institute of Canada Clinical Trials Group MA.17R and NSABP (National Surgical Adjuvant Breast and Bowel Project) B42 adjuvant trials<sup>28,29</sup> (which are randomly assigning patients who are disease free after 5 years of treatment with an adjuvant aromatase inhibitor to extended

hormonal therapy or not). Alternatively, these patients might also be candidates for experimental therapeutic approaches. BCI might help to triage these patients appropriately.

BCI might be advantageous over other contemporary gene-expression signatures because the identification of two rather than three distinct risk groups in each time period (grouping intermediate and low risk together for early recurrence and intermediate and high risk together for late recurrence) potentially eliminates the less actionable intermediate risk category that can account for as many as 40% of patients with oestrogen-receptor-positive breast cancer.<sup>30</sup> In particular, BCI might be useful in the setting of late disease recurrence, because it might provide a much needed means of identifying patients who could be spared extended adjuvant endocrine therapy and its well characterised adverse side-effects. Previously, studies have shown that clinicopathological factors such as nodal status and tumour size are associated with a higher risk of late recurrence;<sup>2,31</sup> however, the results presented here represent a refinement, allowing for individualised assessment of late recurrence risk, and providing statistically significant improvement in prognostic strength above clinicopathological factors. Recently disclosed preliminary studies have suggested that other gene-expression-based assays (EndoPredict,<sup>32</sup> PAM50<sup>33</sup>) have prognostic ability for late recurrence beyond clinicopathological factors. Taken together, these data further validate the clinical use of molecular-based assays for the assessment of late disease recurrence risk (panel).

Analysis of *HOXB13/IL17BR* and MGI, the individual components of BCI, suggested that although each component was prognostic for early recurrence, only *HOXB13/IL17BR* was prognostic for late recurrence. This finding is consistent with results from a correlative study of the MA.17 trial in which *HOXB13/IL17BR* was prognostic for late recurrence.<sup>24</sup> Furthermore, the absence of prognostic strength of MGI for late recurrence is consistent with previous studies suggesting prognostic signatures relying mainly on measurement of proliferation-related gene expression have limited prognostic value for late recurrence.<sup>16,17</sup> Together, these results suggest that the *HOXB13/IL17BR* component of BCI provides additional information and unknown biological functionality beyond tumour proliferation; these *HOXB13/IL17BR* attributes might distinguish BCI from IHC4 and the 21-gene recurrence score.

Additionally, previous studies suggest that high expression of *HOXB13/IL17BR* is not only prognostic, but also predictive of benefit of adjuvant endocrine treatment.<sup>21,24</sup> Thus, the marginal ability of BCI-C, which was developed in the endocrine-treated group of the Stockholm trial, might have been confounded by the dual prognostic and endocrine treatment predictive properties of *HOXB13/IL17BR*.<sup>20</sup> By contrast, BCI-L contains only additive functions of MGI and *HOXB13/*

*IL17BR* and was developed in the untreated group of the Stockholm trial in which clinical outcomes represented the natural history of breast cancer.<sup>21</sup> Findings reported here suggest that BCI-L was spared any confounding effects of the endocrine treatment predictive properties of *HOXB13/IL17BR*, and as a result BCI-L was shown to be the optimum prognostic version of the combination of *HOXB13/IL17BR* and MGI.

Our study has strengths and limitations. Strengths include the use of a standardised quantitative assay (BCI) with methods and analyses that were prospectively defined, and all BCI data were obtained by personnel who were blinded to study outcome or clinical variables. An additional strength was the study's basis in a large contemporary prospective randomised clinical trial with a long follow-up. An important limitation was that in the primary analysis, BCI-C was significantly prognostic as a categorical risk group variable ( $p < 0.0001$ ), but was not significantly prognostic as a continuous variable ( $p = 0.054$ ); thus, analyses relied on a secondary linear combination (BCI-L). Although the numbers of patients categorised into the three risk groups were similar between BCI-C and BCI-L, BCI-L classified a greater proportion of non-recurrent and recurrent patients within the low-risk and high-risk groups, respectively, than did BCI-C; thus, the analyses relied on a secondary linear combination (BCI-L). However, both versions of BCI have been previously reported and validated.<sup>20,21</sup> Additionally, our study included only postmenopausal patients with a follow-up restricted to 10 years. Thus, our findings might be restricted to this population and this timeframe. Also, an unintended selection bias might have applied to our N0 TransATAC cohort, because it consisted of a higher proportion of patients with high-grade tumours and late distant recurrences compared with patients in the N0 non-TransATAC group.

Although BCI was tested against two other recurrence assessment methods, a limitation of this study is that the MammaPrint breast-cancer recurrence diagnostic test has not been assessed in this cohort, and thus it could not be compared in our analysis. Other limitations apply specifically to IHC4. Controversy exists over the variability and comparability of Ki-67 measurements in tissue samples, but rigorous quality assurance standards were used in accordance with recommended guidelines for the measurement of IHC4.<sup>34</sup> Another limitation is the IHC4 model was developed in the same dataset as used in this study, although the sample splitting procedure described previously adjusts for this potential overfitting.<sup>25</sup> Lastly, the absence of a prespecified IHC4 categorical cutoff point might limit the interpretation of its comparative prognostic ability in this study.

In summary, this study has confirmed the independent prognostic ability of BCI in postmenopausal patients with oestrogen-receptor-positive, N0 breast cancer given tamoxifen or anastrozole. Furthermore, our results

### Panel: Research in context

#### Systematic review

We did a systematic review as part of the planning of this study. To identify previous biomarker studies of late recurrence in breast cancer, we searched PubMed for reports published in English between Jan 1, 1980, and Dec 31, 2010, with the terms "late recurrence" and "breast cancer". We retrieved 21 reports, of which we judged 17 to be most relevant. Our systematic review showed there is an unmet need to identify prognostic biomarkers that provide risk estimates beyond standard clinicopathological variables for late disease recurrence in patients with oestrogen-receptor-positive breast cancer.

#### Interpretation

As far as we are aware, this is the first published study to provide a comparative multibiomarker analysis of early and late disease recurrence in a large randomised clinical trial of adjuvant hormonal therapy in postmenopausal patients with oestrogen-receptor-positive breast cancer. Our results show that the breast-cancer index assay (BCI) is prognostic for both early and late distant recurrences in patients with oestrogen-receptor-positive breast cancer and identifies two distinct, clinically actionable populations of patients: those who are at low risk of recurrence and who might be adequately treated with adjuvant hormonal therapy alone, and those who are susceptible to a late recurrence and could be considered for extended adjuvant hormonal therapy or alternative therapy. Clinically, BCI could allow many women with early-stage oestrogen-receptor-positive breast cancer to avoid unnecessary extended antihormonal treatment, and could be an important method to aid the management of residual risk after 5 years of adjuvant hormonal treatment.

support BCI's ability to identify patients at increased risk for late recurrence. Future directions include further examination of the predictive ability of BCI for chemotherapy and extended adjuvant endocrine therapy benefit. From a clinical management view, our results suggest that BCI might have the potential to influence two important decisions in the management of postmenopausal patients with oestrogen-receptor-positive, N0 breast cancer: first at the time of diagnosis and second at 5-year disease-free follow-up.

#### Contributors

DCS was the principal investigator. DCS, YZ, CAS, MGE, and PEG participated in all phases of this study, including design and writing of the biomarker proposal, submission to the ATAC steering and pathology committees for approval, data collection, analysis and interpretation, and preparation of the manuscript. BS participated in data analysis and interpretation and writing of the manuscript. IS did all of the statistical analyses and participated in study design, data analysis and interpretation, and preparation of the manuscript, as did MD and JC. AD, KS, and EL-K participated in data collection and sample analysis. All authors have approved the contents of the manuscript.

#### Conflicts of interest

DCS and MGE are named inventors on a patent to use the breast cancer index, *HOXB13/IL17BR*, and molecular grade index assays to predict

breast cancer outcome. MD and JC have received grant support and lecture fees from AstraZeneca. PEG has received lecture fees from Novartis and GlaxoSmithKline. MGE, CAS, YZ, and BS are employees of bioTheragnostics. AD, KS, EL-K, and IS declare that they have no relevant conflicts of interest.

#### Acknowledgments

We thank the ATAC trialists and pathologists, the ATAC pathology subcommittee, and the ATAC steering committee for their oversight. We thank Ranelle Salunga for technical expertise, and we are indebted to the women who participated in the ATAC trial. MD acknowledges support from the Royal Marsden National Institute for Health Research Biomedical Research Centre.

#### References

- 1 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; **365**: 1687–717.
- 2 Saphner T, Tormey DC, Gray R. Annual hazard rates of recurrence for breast cancer after primary therapy. *J Clin Oncol* 1996; **14**: 2738–46.
- 3 Cuzick J, Sestak I, Baum M, et al, on behalf of the ATAC/LATTE investigators. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol* 2010; **11**: 1135–41.
- 4 The Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol* 2008; **9**: 45–53.
- 5 Regan MM, Neven P, Giobbie-Hurder A, et al, for the members of the BIG 1-98 Collaborative Group and the International Breast Cancer Study Group (IBCSG). Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up. *Lancet Oncol* 2011; **12**: 1101–08.
- 6 Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; **351**: 2817–26.
- 7 Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001; **98**: 10869–74.
- 8 Oh DS, Troester MA, Usary J, et al. Estrogen-regulated genes predict survival in hormone receptor-positive breast cancers. *J Clin Oncol* 2006; **24**: 1656–64.
- 9 Loi S, Haibe-Kains B, Desmedt C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol* 2007; **25**: 1239–46.
- 10 van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; **347**: 1999–2009.
- 11 Wang Y, Klijn JGM, Zhang Y, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005; **365**: 671–79.
- 12 Ma XJ, Wang Z, Ryan PD, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell* 2004; **5**: 607–16.
- 13 Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res* 2010; **16**: 5222–32.
- 14 Desmedt C, Haibe-Kains B, Wirapati P, et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res* 2008; **14**: 5158–65.
- 15 Wirapati P, Sotiriou C, Kunkel S, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res* 2008; **10**: R65.
- 16 Desmedt C, Piette F, Loi S, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clin Cancer Res* 2007; **13**: 3207–14.
- 17 Albain KS, Barlow WE, Shak S, et al, for The Breast Cancer Intergroup of North America. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol* 2010; **11**: 55–65.
- 18 Klein CA, Blankenstein TJF, Schmidt-Kittler O, et al. Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. *Lancet* 2002; **360**: 683–89.
- 19 Schmidt-Kittler O, Ragg T, Daskalakis A, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci USA* 2003; **100**: 7737–42.
- 20 Jerevall PL, Ma XJ, Li H, et al. Prognostic utility of HOXB13:IL17BR and molecular grade index in early-stage breast cancer patients from the Stockholm trial. *Br J Cancer* 2011; **104**: 1762–69.
- 21 Zhang Y, Schnabel CA, Schroeder BE, et al. Breast cancer index identifies early stage ER+ breast cancer patients at risk for early and late distant recurrence. *Clin Cancer Res* 2013; **19**: 4196–205.
- 22 Ma XJ, Salunga R, Dahiya S, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. *Clin Cancer Res* 2008; **14**: 2601–08.
- 23 Ma XJ, Hilsenbeck SG, Wang W, et al. The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer. *J Clin Oncol* 2006; **24**: 4611–19.
- 24 Sgroi DC, Carney E, Zarrella E, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. *J Natl Cancer Inst* 2013; **105**: 1036–42.
- 25 Cuzick J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol* 2011; **29**: 4273–78.
- 26 Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol* 2010; **28**: 1829–34.
- 27 Rutqvist LE, Johansson H. Long-term follow-up of the randomized Stockholm trial on adjuvant tamoxifen among postmenopausal patients with early stage breast cancer. *Acta Oncol* 2007; **46**: 133–45.
- 28 Goss PE, Muss HB, Ingle JN, et al. Extended adjuvant endocrine therapy in breast cancer: current status and future directions. *Clin Breast Cancer* 2008; **8**: 411–17.
- 29 Mamounas EP, Lembersky B, Jeong JH, et al. NSABP B-42: a clinical trial to determine the efficacy of five years of letrozole compared with placebo in patients completing five years of hormonal therapy consisting of an aromatase inhibitor (AI) or tamoxifen followed by an AI in prolonging disease-free survival in postmenopausal women with hormone receptor-positive breast cancer. *Clin Breast Cancer* 2006; **7**: 416–21.
- 30 Kelly CM, Krishnamurthy S, Bianchini G, et al. Utility of Oncotype DX risk estimates in clinically intermediate risk hormone receptor-positive, HER2-normal, grade II, lymph node-negative breast cancers. *Cancer* 2010; **116**: 5161–67.
- 31 Brewster AM, Hortobagyi GN, Broglio KR, et al. Residual risk of breast cancer recurrence 5 years after adjuvant therapy. *J Natl Cancer Inst* 2008; **100**: 1179–83.
- 32 Dubsky P, Brase JC, Fisch K, et al. The EndoPredict score identifies late distant metastases in ER+/HER2- breast cancer patients. *Cancer Res* 2012; **72** (suppl 3): S4–3 (abstr).
- 33 Sestak I, Dowsett M, Sgroi D, et al. Comparison of five different scores for the prediction of late recurrence for oestrogen receptor-positive breast cancer. *Ann Oncol* 2012; **24** (suppl 3): iii29–37 (abstr).
- 34 Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *J Natl Cancer Inst* 2011; **103**: 1656–64.